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A MANUAL OF BACTERIOLOGY

WILLIAMS

A MANUAL

OF

BACTERIOLOGY

BY

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WITH 108 ILLUSTRATIONS

FOURTH EDITION, REVISED AND ENLARGED

PHILADELPHIA:

P. BLAKISTON'S SON & CO.

1012 WALNUT STREET

1905

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PREFACE TO THE FOURTH EDITION.

In the present edition the scope and purpose of former editions of the manual will be found unaltered, and, aside from verbal changes where these seemed desirable, only such modifications as were required to bring the work up to date have been made. The object aimed at in the revision has been to preserve the characteristic features of the work, while introducing those modifications and additions which progress demanded.

Recent advances made it necessary to enlarge the chapters on bacterial poisons and on immunity, and the additional space given to these subjects is justified by their importance not only to the specialist in bacteriology, but to every well-qualified physician as well. The chapter on immunity in particular will be found to be expanded in the matter of immunity proper, and also in regard to the nearly allied processes of the formation of specific agglutinins, precipitins and cytolysins. In the treatment of these subjects the aim has been to present the well-established results of investigations, and to state briefly the conflicting views in regard to their theoretical explanation. For, although Ehrlich's side-chain theory seems to have found in America and some other countries more general acceptance than the other explanations that have been offered, those of Bordet and of Arrhenius are deserving of consideration on account of the important experimental work which has been done in their support, if for no other reason.

Bacteriology is not a subject which can be learned from books alone or without instructors, and the manual is intended to supplement laboratory work and teaching, but not to supplant

these. Still, methods of cultivation and examination are given in some detail.

Dr. Williams was unable, for a number of reasons, to revise and correct the book himself. The entire responsibility for the present edition (including the chapters by Dr. Clinton and Dr. Carpenter) rests with the reviser.

B. MEADE BOLTON.

WASHINGTON, D. C., September, 1905.

PREFACE TO THE THIRD EDITION.

The plan used in the preceding editions of this manual has been followed in the preparation of the present one. The only departures have been in the insertion of a short historical sketch and the freer use of references to original articles and reviews. It is hoped that these features will assist in arousing the interest of students. As far as possible, reference has been made to articles in American and English journals likely to be easy of access. Besides the ones just named, numerous additions have been made which the recent advances in our knowledge have rendered necessary. Most of the illustrations of apparatus are new. The photomicrographs also are new and original with a few exceptions noted in the text. It is probably needless to say that none of them were retouched. The writer is indebted to the Gratwick Laboratory of Buffalo for the use of its facilities in making these photographs.

BUFFALO, NEW YORK, August, 1903.

PREFACE TO THE SECOND EDITION.

Although there has been no lack of works on bacteriology, it seemed to the writer that there was still a field open for one which sought to give the portions essential to medical science in a concise manner. It is gratifying, therefore, that the first edition of this little book should have been exhausted so soon.

Whether wisely or not, it is a fact that many medical schools require their students to absorb an amount of knowledge that taxes the brain to the utmost. While such conditions remain, the need is urgent for presenting what is taught in the accessory branches in as condensed a form as is consistent with a clear understanding of their great fundamental principles. It is mastery of such principles, after all, which is the object of a course in bacteriology, for they are essential to a correct understanding of most of the other branches. After that has been accomplished (including the applications of bacteriology to diagnosis), it must be admitted that other branches deserve a larger amount of the student's time. This may be said without meaning to minimize the importance of bacteriology in the training of a physician. In the opinion of the writer it is neither possible nor desirable that every graduate should be a trained bacteriologist. However, no instructor can hope to bring the principles above mentioned home to his classes except by laboratory work. Very little attempt has been made to outline the program of a laboratory course, as that will always need to be planned according to the circumstances under which it is given.

The purpose of this book is to give in the smallest possible space the facts which a physician must know, with some of those

which it is desirable that he should know, and a little of that which he may learn if his needs or inclinations lead him to go further. It is acknowledged, however, that, in deference to precedent, this purpose has not been carried to its fullest extent. Much time has been spent on the index, in order to make the contents quickly accessible. It is a source of regret to the writer that the additions which the revision seemed to demand have made the present book a little larger than the first edition.

Buffalo, New York, June, 1901.

PREFACE TO THE FIRST EDITION.

In this manual the writer has endeavored to describe the laboratory technique which the beginner must follow, and at the same time to give a concise summary of the facts in bacteriology most important to the physician. In preparing a work of this character, which claims to be nothing more than a compilation, the standard text-books were necessarily consulted freely. On account of the need for brevity it has, in most cases, been impossible to mention authorities.

The writer is glad to have this opportunity to acknowledge his obligation to the works of Sternberg, Flügge, Günther, Eisenberg, Abbott, W. H. Park, Muir and Ritchie, Vaughan and Novy, and McFarland; and to numerous papers by Professor Welch and others. It is thought that the chapters on Germicides and Surgical Disinfection, by Drs. Thomas B. Carpenter and Marshall Clinton, will be useful not only for the information presented in them, but especially in correlating that information with the facts of bacteriology.

BUFFALO, NEW YORK, October, 1898.

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INTRODUCTION.

Anyone who has not himself worked in a bacteriological laboratory finds it difficult to form a vivid conception of what bacteria are like, because among the familiar animals and plants there are none with which a close comparison can be made. Of the common organisms, perhaps ordinary yeasts and moulds are most like the bacteria. Yeasts and moulds, as everyone knows, grow on bread, cheese, meat, syrups and the like. They flourish in moist and dark places, as do mushrooms, puffballs and the other fungi. All these fungi, appearing so different in some respects, are alike in one particular, which is the absence of the green color that we are apt to think of as being the essential feature of vegetation. Plants that are green owe their color to a substance called chlorophyll. Upon the properties of this substance one of the most fundamental facts in biology depends. Under the influence of sunlight, by means of chlorophyll, plants are able to use as food the carbon dioxide which is always present in the atmosphere in small amounts. Although carbon dioxide is one of the most simple and stable of compounds, the union of its component elements is broken by the plant, and they are employed in the formation of other much more complex and unstable compounds, such as starch and cellulose, which enter into the plant's structure. The work of plants, it will be noticed, is, in the main, precisely the reverse of that performed by animals. Animals take the unstable carbohydrates with high potential energy, such as starches and sugars, as food, and exhale the stable carbon dioxide from the lungs. At the same time the animal receives the benefit of the energy resulting from

the oxidation of the carbohydrates, which may appear indirectly in the form of nervous or muscular activity or warmth.

Those plants that are devoid of chlorophyll are compelled to some extent to use the same kinds of food as animals. They are unable to decompose carbon dioxide (in most cases), and procure their nourishment from substances derived from the dead or living bodies of other plants or animals. Since they have no chlorophyll, light is of no advantage to them, and is often a positive detriment. Bacteria contain no chlorophyll, and consequently are unable to decompose carbon dioxide and to use it as food.*

There is another well-known property, possessed by yeasts especially, which may be useful in explaining the work done by bacteria. It is a fact of every-day observation that, when ordinary yeast grows in fruit juice or other fluids containing sugar, alcohol and gas are formed. It not only appears that bacteria sometimes form alcohol and gas from sugar, but that with different kinds of bacteria and different kinds of food material a great number of substances are formed, some of which are powerful poisons. In most, if not in all, of the diseases caused by bacteria such poisons are produced within the living body of the affected individual, and the symptoms of the disease and the changes produced in the body are certainly due to these poisons, as a rule, rather than to the direct action of bacteria.

The extreme smallness of the bacteria prevents us from seeing them as individuals without the aid of the microscope, although great numbers of them taken together may form a plainly visible mass of growth. When they are examined with the microscope they appear as little, round, rod-shaped or curved bodies, which may be likened to so many periods, dashes and commas. It is at once perceived that each bacterium is an individual by itself,

* See Part II., Chapter I.

and that it consists of a single cell, not of an aggregation of cells, as do most of the common plants and animals.

Under favorable conditions bacteria undergo rapid multiplication. The individuals in some forms divide into two cells, in other forms into four cells, in others again into eight cells simultaneously. The process takes place by direct cell division, and is called *fission*.

Under certain conditions, bright, glistening bodies make their appearance in certain bacteria, and become larger and larger, while the cells in which they develop break up into fine fragments. These bodies are called *spores*, and represent a resting stage in some respects resembling the seeds of higher plants. They have much greater resisting power against injurious influences than is possessed by the growing or *vegetative forms*. There are spores that can withstand boiling for hours, but fortunately that is not true, as far as we know, of the spores of any of the bacteria that produce disease. The earlier investigators observed the appearance of bacteria in nutrient infusions which they had endeavored to sterilize by heat. They looked upon this fact as indicating the possibility of *spontaneous generation*, and it furnished the chief support of that theory. Probably their infusions contained very resistant spores, and were in reality not sterile.

From these facts a definition for bacteria may be formulated.

Bacteria (Greek βακτήριον, meaning a little stick) *are extremely minute, unicellular plants, which have no chlorophyll, and which divide by fission.* They are sometimes called *schizomycetes*. In every-day language they are known as *microbes*, and also as *germs*. They are generally classed with the fungi. In some respects they seem quite closely related to the algæ or simplest green plants, and, on the other hand, they have strong points of likeness with some of the unicellular animals belonging to the infusoria.

Bacteria are divided into two great groups:

I. The lower bacteria include these forms which are of most importance at present, and consist of—

Micrococci, or cocci (singular, coccus)—spherical forms.

Bacilli (sing., bacillus)—long and straight, or rod-shaped bacteria.

Spirilla (sing., spirillum)—consisting of spiral filaments like the turns of a corkscrew, or parts of spirals shaped like commas.

Micrococci. Bacilli. Spirilla.

FIG. 1.

II. The higher bacteria, which consist of long filaments made up of segments more or less united. In some of these the filaments show dichotomous branching. This group is more fully discussed under the non-pathogenic bacteria, Part III. A few of them are pathogenic.

The extreme smallness of the bacteria is hard of comprehension. We may say, of most of them, that from 5,000 to 25,000 placed end to end would make a line about an inch in length. When one touches a growth of bacteria with the sterilized platinum wire and spreads the tiny portion that adheres to the wire upon a slip of glass, it is found upon examination with the microscope that the bacteria left on the glass may be compared to the stars in the sky, the grains of sand on the shore, or any of the other standards for numbers that are nearly beyond computation.

It is well known that bacteria are present on most of the objects about us. They occur on the skins of men and other animals, as well as in the mouth, stomach and intestines, and on most of the surfaces of the body that open to the external world. They are found in the water of rivers and lakes, and in the ocean. They appear in the soil down to a depth of several feet. They float in the air, except at high altitudes and over the ocean. Nansen found bacteria on the ice of the Polar sea. Investigators have even reported finding them fossilized, indicat-

ing, as we might expect, that they existed at remote periods in the earth's history. But the vast majority of them are entirely harmless as far as we are concerned, and many of them are indispensable in maintaining the balance existing between the different kinds of living things.

Were it not for the putrefactive and nitrifying bacteria the dead bodies of plants and animals would lie practically unchanged where they fall, and the fertilization of the soil necessary for the life of most plants, by means of substances derived from such dead material, would cease.

In northern Siberia the bodies of the extinct species of elephant called mammoths have been found imbedded in frozen soil where they appear to have lain for thousands of years. In this case the growth of putrefactive bacteria has been prevented by cold, as in the modern refrigerator or cold-storage plant.

Some bacteria have been made to do work in industries, like the bacilli whose growth in cream imparts an agreeable flavor to the butter and cheese.

Other bacteria are also made use of in the manufacture of vinegar.

Some kinds of bacteria of the soil are employed to take nitrogen from the atmosphere, adding nitrogen compounds to the soil, which take the place of chemical fertilizers.

The study of bacteria has led to the understanding of many hitherto unexplained facts. The unaccountable development of a moist, brilliant red deposit on bread and other articles of food, which was formerly believed by the superstitious to be blood, deposited by some miraculous agency, we know to be due to the growth of a common organism (bacillus prodigiosus). The emission of light by decaying substances when seen in the dark is caused by bacteria as well as other organisms.

It seems that in some cases in which death has been attributed to the suction of air into the veins, because air appeared to be present inside the heart, the air was in reality a gas, formed

by certain bacilli that invaded the body just before or just after death (bacillus aërogenes capsulatus).

Woodhead tells us that some savages are in the habit of smearing the soil of certain localities upon their arrows for an arrow-poison, which is intelligible in the light of the fact that soil often contains the bacilli of tetanus (lockjaw).

The comparatively small number of species of bacteria that cause disease are the ones that interest us most, and are those which have been most carefully studied. The necessity that falls upon bacteria, in common with other fungi, to derive their food from organic matter makes it easy to understand that they should frequently exist as parasites upon living animals and plants. Pear-blight and some other diseases of plants are caused by bacteria. We find that frogs, birds, cattle and a great number of animals besides men suffer from diseases produced by bacteria.

When bacteria are placed upon slips of glass they may be studied with the microscope while alive. Some of them when living are motionless; others wriggle vigorously. Some dart about like minnows in a stream, or they make their way slowly across the field of the microscope like a boat that is being sculled from the stern. By proper methods it can be shown that the movements are effected through one or more fine, hair-like processes called flagella.

Often it is expedient to study bacteria after drying them on slips of glass, when they may be made more conspicuous by giving them an artificial color (staining). Some of the substances of which they are composed readily absorb certain dyes. For this purpose the aniline dyes are used, and their employment has been one of the important factors in making progress in bacteriology possible.

With the microscope alone it is not usually practicable to distinguish accurately between various kinds of bacteria. Micrococci, for instance, which are, in reality, extremely

different, may look very much alike. The differences usually become apparent when the bacteria are grown artificially. The cultivation is done for the most part in test-tubes containing some material which furnishes suitable food. The nutrient materials most used are meat-extract and peptone, which, dissolved with salt in water, constitute *nutrient bouillon*. Ordinary *gelatin*, or a vegetable gelatin called *agar-agar*, may be added to the bouillon when a solid *culture-medium* is desired. Before these substances can be used for the cultivation of bacteria all other bacteria which they may contain must be destroyed by heat.

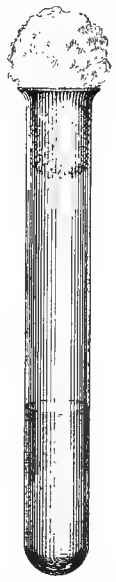

FIG. 2.—TEST-TUBE CONTAINING CULTURE-MEDIUM.

Finally, the effects of bacteria in bringing about disease may be tested on the lower animals. The proof that a particular species of bacteria causes a particular disease cannot be considered complete unless the disease can be reproduced by introducing these bacteria into some animal.

Bacteriological Literature.—The student who wishes to pursue bacteriological study in any direction farther than it is possible for the limits of a short manual to go, may, besides consulting the large text-books, and weekly medical journals, obtain much assistance from technical periodicals. The *Journal of Experimental Medicine*, *Journal of Medical Research*, and the *Journal of Infectious Diseases*, published in this country, and the English *Journal of Pathology and Bacteriology* and *Journal of Hygiene* will give a great deal that is valuable.

A reading knowledge of German and French is very desirable. The *Centralblatt für Bakteriologie, etc.*, a German periodical, and the *Bulletin de l'Institut Pasteur*, published semimonthly

in Paris, contain abstracts of most of the important researches made in all parts of the world. The *Annales de l'Institut Pasteur*, the *Zeitschrift für Hygiene*, and the *Archiv für Hygiene* contain many original articles on bacteriological subjects.

The whole literature of any specified subject in bacteriology can be most conveniently found in *Baumgarten's Jahresbericht der Mikroörganismenlehre.*

Those who are interested in agricultural bacteriology should read the experiment station records and the various bulletins issued by the Department of Agriculture of the United States. They can usually be obtained upon application to the Department at Washington, D. C. The bacteria that produce disease in domestic animals are described in Dr. V. A. Moore's book, "The Infectious Diseases of Animals," Taylor & Carpenter, Ithaca, N. Y., 1902, and in the "Special Report on the Diseases of Cattle," United States Department of Agriculture, 1904.

Historical Sketch.—The remarkable growth of mechanical and industrial enterprises which the last half century has witnessed is held to be characteristic of it. The world justly takes pride in its achievements along these lines. Nearly all that we know of bacteria and the part they play in producing disease has been learned during the same period. It is but fair to say that the rapid growth of this knowledge has been equally characteristic of the age.

Nevertheless many facts were known long ago, and even by the ancients, which were effective in directing the thought of later years. The epidemic nature of certain maladies was naturally among the earliest of these to be noticed, and was, even until recently, attributed to the influence of gods, demons, or other supernatural agencies. The superstitions and crude beliefs of the past gave rise to a mass of grotesque theories and fanciful speculations. But with all this we hear of certain beliefs and practices which plainly foreshadowed those of the present day. Latin writers nearly two thousand years ago recorded a relation between insects and malaria which has but lately been proved and explained. The treatment of lepers by

the Hebrews resembles that now in vogue: "He is unclean: he shall dwell alone; without the camp shall his habitation be" (Lev. XIII., 46). There is, in fact, much in the laws of Moses that points to some knowledge of the nature of infections. "This is the law, when a man dieth in a tent: all that come into the tent and all that is in the tent shall be unclean for seven days. And every open vessel which has no covering upon it shall be unclean" (Numb. XIX., 14, 15).

"Everything that may abide the fire, ye shall make it go through the fire, and it shall be clean" (Numb. XXXI., 23).

In Homer we read of Ulysses, that, having slain his wife's troublesome suitors:

> "With fire and sulphur, cure of noxious fumes,
> He purged the walls and blood-polluted rooms" (Pope's Odyssey).

The massive aqueducts of the Romans still remain to testify that they understood the importance of a pure water-supply.

In Rome there were also sewers for the disposal of drainage, while the Cretans and Assyrians used sewerage systems hundreds and even thousands of years before.

About the fourteenth century we find quarantine against infectious diseases, plague in particular, practiced by certain Italian cities; and the word "quarantine" came into use from the fact that the period of detention was about forty days (Ital. *quarantina*).*

Leeuwenhoek, a citizen of Delft, in Holland (1632–1723) appears to have been the first who actually saw bacteria. Yeast-cells he certainly observed, besides making many other contributions of great value to biology. Leeuwenhoek produced admirable lenses of high magnifying power, and described what he witnessed with singular accuracy and enthusiasm.

---

* J. M. Eager. The Early History of Quarantine. *Yellow Fever Institute Bulletin*, No. 12. U. S. Marine Hospital Service.

Even before this time men had sought to explain the phenomena of infectious diseases by supposing the body to have been penetrated by minute parasites,—for example, worms. The spread of such diseases through a community from a single center could readily be accounted for by the multiplication of a contagious element, itself alive (*contagium vivum*). With increasing knowledge of the abundance of microscopic life these speculations took firmer hold. But long before their truth was finally demonstrated great advances were made in the prevention of infectious diseases. Much honor is due the clinicians, whose accurate observations and foresight accomplished important results at an early day, working with what now seems a very meagre knowledge of the facts.

The production of immunity from small-pox by inoculation was first practiced in oriental countries. The method had long been in use in the East, when in 1718 it was brought to the notice of Europeans by Lady Montagu, wife of the English ambassador at Constantinople. The procedure consisted simply of the introduction of the virus of small-pox by puncture of the skin. An attack of small-pox resulted, which was usually much milder and far less dangerous than the natural disease.

Lady Montagu stated in a letter: "Every year thousands undergo the operation; and the French ambassador says pleasantly that they take the small-pox here by way of diversion, as they take the waters in other countries." The mild attacks that followed inoculation were, however, just as contagious to other persons as the natural disease, so that the dangers of this practice to the community were very great.

A much better method was found in vaccination. At this time a belief was current among farmers that a mild form of disease, called cow-pox, acquired by milkers, furnished protection against small-pox. This belief was investigated and introduced to the world by Edward Jenner. In 1796 he inocu-

lated his first patient with cow-pox. In a few years the practice of vaccination spread to all parts of the world.*

It was introduced into the United States by Dr. Benjamin Waterhouse of Harvard. President Thomas Jefferson was active in bringing it into general use, especially in the South.

As early as 1847 Semmelweis of Vienna attributed the origin of puerperal fever to poisons carried by the fingers of physicians and students, whose hands had been soiled in the dissecting room. To this he was led by the death of a friend from pyemia following a dissection-wound. He noted the similarity of the course of his friend's case with cases of puerperal fever. He advocated washing the hands of the attendant in solutions of chlorine or chloride of lime, in addition to cleansing them with soap and water.

The cause of puerperal fever was still unknown. Endeavors to connect it with atmospheric influences and the like had been unsuccessful. During the seventeenth and eighteenth centuries it had been attributed to the absorption of milk into the blood from the breasts. Semmelweis stood his ground in spite of opposition and ridicule, though he somewhat modified his doctrine. His views agree substantially with the practice of the present day, which they have greatly influenced.

During the same period similar ideas were advanced by Dr. Oliver Wendell Holmes in the United States. His paper on "The Contagiousness of Puerperal Fever" appeared in 1843. A lively controversy lasting several years was provoked, in which Holmes defended his position with great vigor. His admirable literary style served him effectively.†

In the first half of the nineteenth century, with improved microscopes, knowledge of minute living things grew rapidly,

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* See the works of Edward Jenner by Dock. *New York Medical Journal.* Nov. 29 and Dec. 6, 1902. Also Dulles. The History of Vaccination. *Philadelphia Medical Journal.* May 30, 1903.

† See O. W. Holmes. Medical Essays. Houghton, Mifflin & Co., 1889.

chiefly with respect to infusoria and other relatively large forms. In 1840 Henle described the part played by micrcörganisms in producing disease in terms surprisingly in accord with views held at the present time. His deductions were based almost entirely on knowledge of the general nature, spread and course of infections. So, too, Villemin anticipated the discovery of the bacillus of tuberculosis, for he transmitted the disease to animals, by inoculating them with material from cases of tuberculosis in man.

The key to exact knowledge of the microörganisms of disease was finally discovered in the study of fermentation. No better illustration could be found of the possible value to mankind which may lie in any addition whatever to the common stock of knowledge. The study of bottles of bad-smelling broth would have seemed, fifty years ago, a most unpromising beginning for the discovery of the causes of cholera, plague, and the like, or for an antitoxin for diphtheria.

Studies on Fermentation and Spontaneous Generation.— Two observers (Schwann, Cagniard-Latour, 1837) almost simultaneously stated the proposition that yeast cells were living organisms, and that the fermentation of solutions of sugar was due to their growth. From this time ensued a controversy which lasted more than thirty years. The agency ascribed to yeasts was energetically denied by many, prominent among them Liebig; while it was sustained with vigor by others. The latter extended the original conception to include other sorts of fermentation and the putrefaction of albuminous material. Different kinds of fermentation, with different products, such as acetic acid and butyric acid, were ascribed to the growth of different kinds of microbes.

These microbes were found to be fungi of various sorts, and chiefly one or another variety of bacteria. The most celebrated among the students of fermentation was Pasteur, the simplicity

and kindness of whose character excite our admiration equally with his devotion to his work.*

Before the nature of fermentation was understood the possibility of spontaneous generation had been universally admitted. When vermin of various sorts appeared in putrefying material the conclusion was drawn that they had their origin directly from it. Although that had long since been disproved in the case of large organisms like worms and frogs, still, as late as the middle of the last century, it was held by many to account for the swarming microscopic life found in fermenting fluids. A flask of meat broth left exposed to the air will after a few days contain countless tiny living things, chiefly bacteria. Pasteur and his supporters showed that these bacteria were the progeny of others already in the flask or which had fallen in from the air.

When the flask of broth was boiled, no development of organisms took place, if the entrance of germs from the atmosphere was prevented. The latter was accomplished by such devices as heating the air, passing it through sulphuric acid, using a flask with a long twisted neck or by plugging the flask with cotton (Schröder and Von Dusch).

To prove that boiling had not made the fluid unfit for the growth of organisms, air was subsequently allowed to have access to it without such precautions, when putrefaction took place in the usual manner.

At the same time it was demonstrated not only that bacteria are present in all fermenting and putrefying substances, but that they exist wherever there is animal life or vegetation.

These principles underlie the methods used daily for the preservation of meat, fruit and vegetables, in the household and in factories.

Although even boiling occasionally failed to prevent fermentation, investigators came with practice to have a smaller

* See Louis Pasteur. His Life and Labors. By His Son-in-Law. Translated by Lady Claude Hamilton.

number of failures. Such failures it was shown were due to the presence of the resistant forms of the organisms called spores previously alluded to which some bacteria assume. The true nature of spores was recognized later by Cohn. Pasteur found that exposure to steam at temperatures sufficiently high above the boiling point would destroy the most resistant microbes and their spores.

The controversies over fermentation and putrefaction lasted almost until the present day. They have been productive of numerous benefits to the arts and manufactures. But, what is of more importance to our subject, they led to a vastly better understanding of diseases producing microörganisms. The study of bacteria has been pursued with such vigor in the last twenty-five years in fact that most of what we know concerning the bacteria of disease has been learned during this period, and advances are still constantly being made.

The discussions concerning fermentation and putrefaction were still going on when Lister made his brilliant deduction that suppuration and septic processes in wounds were a species of fermentation (1867). From this came the antiseptic and aseptic methods of operating and of dressing wounds, which have made possible the wonderful results of modern operative surgery.*

In 1834 the parasite of itch (*Acarus scabiei*, the itch mite, an arachnid, related to the insects) was discovered, and the cause of one contagious malady determined.

Quite early in the nineteenth century also the relatively large fungi of thrush and some of the parasitic skin diseases were discovered. The bacilli of anthrax, which are also relatively large, were seen in the blood of animals by Pollender in 1855 and Davaine in 1863.

Davaine produced anthrax in animals by injecting into them

* See Dr. Roswell Park. History of Medicine.

blood containing anthrax bacilli. But complete proof that these bacilli were the cause of the disease required that they should produce it when injected alone and when freed from the smallest trace of material derived from the first diseased animal. Unless these conditions were complied with, some other material, for example an enzyme or ferment, might be supposed to be carried from the first to the second animal and to be the real cause of the disease. For this purpose it was necessary to cultivate the bacilli in nutrient fluids, such as meat broth, as was done by Pasteur. It then became possible to demonstrate that their properties could remain unaltered after being grown in successive generations on different lots of broth. As bacteria of two or three species were often encountered in mixtures, it became most important to secure a method by which the different species could be separated from one another and be propagated as separate "pure cultures." This was done successfully by diluting such mixtures greatly, so that a drop planted in a new tube of broth should contain only a single organism. The growth ensuing would of course consist of the same kind of organism exclusively. Such procedures were uncertain and very laborious.

Koch introduced in 1881 his method of separating bacteria by "plating," described below (Part I., Chapter V.), and this is probably the most important single contribution to bacteriological technique which has ever been made. Koch also brought solid culture-media into general use by employing gelatin. Other important technical improvements of the same period were the adoption of the illuminating apparatus of Abbé and immersion objectives, and of aniline dyes for staining bacteria and making them visible (Weigert and Ehrlich). Beginning with the bacillus tuberculosis described by Koch in 1882, a number of pathogenic bacteria were discovered during the ensuing years in rapid succession.

The application of the newly-gained knowledge concerning

the bacteria causing infectious diseases to the prevention and cure of these diseases was begun almost immediately by Pasteur. A few facts existed to guide the direction of the research. It had been known even in ancient times that one attack of an infectious disease, such as scarlet fever, may confer immunity from subsequent attacks.

The protection against small-pox which was furnished by vaccination also was suggestive, although the mechanism by which this protection came about was not understood.

Pasteur worked on the theory that immunity from a disease would probably be secured by producing a mild attack of the disease. Such a mild attack might be expected to follow if a susceptible individual were inoculated with microbes of lowered virulence. Various methods were employed to reduce the virulence of bacteria, notably cultivation at high temperatures (43° C.). Pasteur was able to produce immunity against a number of the diseases of the lower animals. His method of inoculating sheep and cattle against anthrax is widely and successfully used. A somewhat similar principle has led to the preparation of a vaccine for the disease of cattle called "black leg," and such vaccine is now distributed gratuitously to farmers by the United States government. Inoculation of human subjects with the attenuated virus is used for hydrophobia. This method also was devised by Pasteur.

The discovery of antitoxins for infectious diseases (see Part II., Chapter VII.) we owe to Behring. This portion of our subject belongs entirely to the present day, and is now being studied with great energy.

Allusion has already been made to moulds and other microscopic parasites whose nature makes their study almost inseparable from that of the bacteria. In this class also belong the primitive forms of animal life (Protozoa) which are the causes of amebic dysentery (Lösch, 1875) and malaria (Laveran, 1880). The disease of cattle called "Texas fever" is also

caused by a protozoön. Theobald Smith in the United States discovered that the parasite of Texas fever is conveyed from one animal to another by the cattle-tick. Since then it has been shown (by Manson, Ross and others) that malaria is conveyed from a person having the disease to one not affected by means of mosquitoes. It has also been shown by Reed and Carroll that a similar relation exists between mosquitoes and yellow fever. The part played by flies and other insects in carrying disease germs is still receiving active attention and the future may show that these play a most important part in diseases other than those already mentioned.

In 1903 Novy and McNeil succeeded in making pure cultures of pathogenic protozoa grow in tubes, in nearly the same way that cultures of bacteria are propagated (see Appendix).

It is encouraging to reflect that the progress of bacteriology has been made by gradual and logical steps. The great discoveries have not been lucky accidents, but have been worked out patiently and with deliberation.

PART I.

CHAPTER I.

EXAMINATION OF BACTERIA WITH THE MICROSCOPE, INCLUDING METHODS OF STAINING.

The Microscope.—The microscope consists of a tubular body which carries the optical parts, and which can be raised or lowered for focusing. It is a matter of convenience to have three lenses attached to the body of the instrument by means of a triple nose-piece, which permits any objective to be turned into the optical axis at will. But a low power dry lens and an oil-immersion objective are all that are essential for studying the bacteria. The eye-piece slips into the upper and opposite end of the body or tube. The arrangements for focusing consist of a rack and pinion which accomplish the coarse adjustment, and a more delicate fine adjustment. The stage, upon which the objects to be examined are placed, has an opening in the middle. In this opening an iris diaphragm and Abbé condenser are inserted. The iris diaphragm enables one to alter the size of the opening as desired. Beneath the stage is a movable mirror, of which one side is plane and the other concave. All of these parts are supported on a short, heavy pillar, which is fixed in the horseshoe-shaped base.

The essential parts of the microscope are, of course, the eye-piece (German, *Ocular*), and the objective. Objectives are variously designated by different makers, for instance, some use letters, A, B, C, etc., others use numbers, 1, 2, 3, etc., others again give the focal distance, as $\frac{2}{3}$ inch, $\frac{1}{4}$ inch, $\frac{1}{2}$ inch, etc.

In bacteriological work a rather "low power" $\frac{2}{3}$ or $\frac{3}{4}$ inch objective, and a high power $\frac{1}{12}$ inch oil-immersion objective are needed. A $\frac{1}{4}$ or $\frac{1}{6}$ inch dry objective may also occasionally

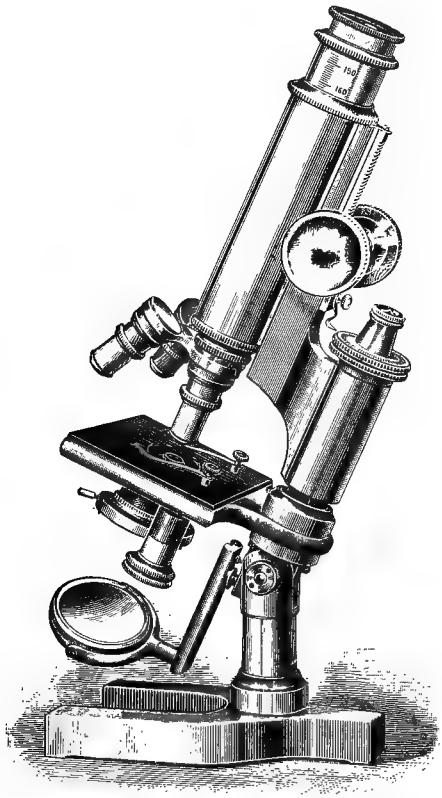

FIG. 3.—MICROSCOPE.

be useful. The magnification with the $\frac{2}{3}$ or $\frac{3}{4}$ inch objective is about 75 to 100 diameters; with the $\frac{1}{4}$ to $\frac{1}{6}$ inch 300 to 500 diameters; with the $\frac{1}{12}$ immersion 750 to 1000 diameters. The magnification varies according to the eye-piece used, as well

as with the objective. A 1 inch and 1½ inch eye-piece (Zeiss No. 2 and No. 4) serve well for most purposes. The eye-pieces are usually named arbitrarily, like the objectives. In using the $\frac{1}{12}$ immersion objective a layer of thickened oil of cedar-wood is placed between the lower surface of the objective and the upper surface of the glass covering the object under examination. The oil must be wiped away from the surface of the objective when the examination is finished. For this purpose the soft paper sold by dealers in microscopic apparatus serves admirably. Care must be taken not to scratch the lower surface of this objective. Oil of cedar-wood furnishes a medium having nearly the same refractive index as the glass of the lens and the

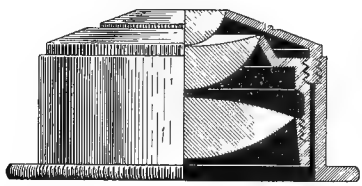

FIG. 4.—ABBÉ CONDENSER.
On the right side the figure gives a sectional view.

glass on which the object is mounted, and it obviates the dispersion of light which takes place when a layer of air is interposed between the objective and the object, as happens with the ordinary dry lens. This objective is used in connection with the Abbé condenser, which consists of two or three lenses combined so as to focus the rays coming from the plane mirror upon the object. The condenser gives a very intense illumination over a very small field. The condenser is not necessary excepting with the oil-immersion objective. If it is used with the other objectives the illumination must be regulated by lowering the condenser, closing the diaphragm more or less, and substituting the concave for the plane mirror. It is to be remembered

that more depends upon securing a distinct picture than upon a very high magnification of the object.

The microscope should be placed in front of the observer on a firm table. The observer should be able to bring the eye easily over the eye-piece when the tube of the microscope is in vertical position. Daylight should be employed if possible, but not direct sunlight. When artificial illumination is necessary, an ordinary lamp, a Welsbach burner or an incandescent electric light may be used. It is best to modify the artificial light by inserting a sheet of blue glass between the light and the mirror.

In order to focus upon any object, having first secured a satisfactory illumination with the mirror, it is best, beginning with the low power and using the coarse adjustment for focusing, to bring the objective quite close to the object, and then, with the eye in position, to raise the tube until the object comes into focus. The exact focusing is done with the fine adjustment. The observer should keep both eyes open when using the microscope, and should be able to use either eye at will.

All measurements of microscopic objects are expressed in terms of a micromillimeter. This is one-thousandth of a millimeter (0.001 mm.), which is about $\frac{1}{25000}$ of an inch. It is generally called a micron for short, and is denoted by the Greek letter μ. For example, $5\ \mu = 0.005$ mm. $= \frac{1}{5000}$ inch.

The Preparation of Specimens of Bacteria for Examination with the Microscope.—The substance under examination is placed upon thin slips of glass called cover-glasses. The material is spread over the cover-glass by means of a platinum wire which has been fixed in a glass rod about six inches long. Such a platinum wire is used constantly in doing bacteriological work. It is the tool by means of which one is able to handle bacteria with impunity. It serves, in fact, as a kind of additional finger. The platinum wire must be stiff enough not to bend too easily, and yet it should not be

so large that it will not cool rapidly after heating. A good size for most purposes is number 23, American wire gauge (Brown & Sharp). The wire may be straight throughout its length, or the tip may be bent to form a loop (German, *Oese*). It is well to follow, from the beginning, certain rules which make the use of the platinum wire safe and accurate. Every time it is taken into the hand and before using it for any manipulation heat it in the flame of a Bunsen burner or an alcohol lamp to a red heat; and always, *after using*, and *before putting it down, heat it again to a red heat.* If the needle is wet, it should be dried by holding it near the flame in order to avoid the "sputtering" which occurs if it is plunged at once into the flame. This precaution is

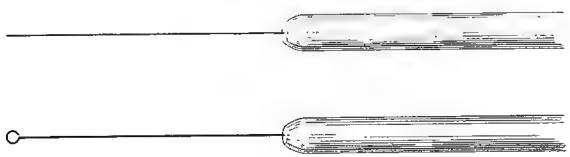

FIG. 5.—STRAIGHT PLATINUM WIRE AND PLATINUM WIRE LOOP.

especially called for when the wire has been dipped in milk or other substances containing oil. When the needle "sputters," as it is called, from too rapid heating, particles that have not yet been sterilized may be thrown some distance. On no account should the needle touch any object other than that which it is intended it should touch. With such a platinum wire, which has been properly sterilized, one can easily remove portions from a culture of bacteria, or from a fluid in which bacteria are supposed to be present. The glass rod in which the platinum wire is fixed should be held between the thumb and forefinger of the right hand like a pen. (For the manner of holding test-tubes, see page 73.)

The Hanging-drop.—Living bacteria may be studied with

the microscope while suspended in some fluid substance. This is accomplished by means of a hanging-drop. In order to prepare a hanging-drop for examination a clean cover-glass is held in the forceps and a small drop of the fluid to be examined is spread thinly over the center of it by means of a platinum needle which has just previously been heated in a flame and allowed to cool. The needle should again be sterilized in the flame. When cultures on solid media are to be examined, a small particle may be mixed with a drop of sterilized water or bouillon which has first been placed in the middle of the cover-glass. The cover-glass should have been carefully cleaned and sterilized over the flame. The cover-glass with the thin drop of fluid material held in sterilized forceps is now to be inverted over a sterilized glass slide, which has a concavity ground in the

FIG. 6.—DIAGRAM OF THE HANGING-DROP.

middle of it. Around the concavity, the slide should be smeared with vaseline. In this manner a small, air-tight chamber is made. This preparation may be put upon the stage of the microscope. A good dry lens, if of sufficiently high power, is more convenient for examining the hanging-drop than an oil-immersion. If the latter be used, having placed a drop of cedar-oil on the center of the cover-glass, and a good light having been secured, the oil-immersion objective should be brought down upon this drop of oil. The beginner often experiences difficulty in focusing upon a hanging-drop. It is well to shut off most of the light by means of the iris diaphragm. Often it is well to secure the focus roughly upon the extreme outer edge of the chamber, or to find the edge of the drop of fluid with the low power and then to focus upon this edge with the oil-im-

mersion objective. Above all things guard against breaking the cover-glass by forcing the objective down upon it. The motility of certain bacteria is one of the most striking phenomena to be observed in the hanging-drop. It is not to be confused with the so-called "Brownian movement" which is exhibited by fine particles suspended in a watery fluid. It is well for the beginner to observe the character of the Brownian movement by rubbing up some carmine in a little water, and with the microscope to study the trembling motion exhibited by these particles of carmine. It will be noticed that, although the particles oscillate, no progress in any direction is accomplished unless there are currents in the fluid. Such currents might give rise to the impression that certain bacteria possessed motility when they were, in fact, powerless to move of themselves. In the hanging-drop the multiplication of bacteria can be studied, the formation of spores and the development of spores into fully formed bacteria. The hanging-drop has recently been put into service for the demonstration of the so-called serum-reaction with the bacillus of typhoid fever. Sometimes bacteria must be watched in the hanging-drop for hours, or even days, and it may be necessary to keep it at the temperature of the human body for this length of time. Various complicated kinds of apparatus have been devised for this purpose, but they are needful only with special kinds of work. When the hanging-drop preparation is no longer required, the slide and cover-glass should be dropped into a 5 per cent. carbolic acid solution and afterward sterilized by steam.

Hanging-block preparations, which were introduced by Hill,* consist in the use of a cube of nutrient agar instead of a drop of fluid. Bacteria are distributed on the surface of the agar, which is then applied to a cover-glass, and mounted like a hanging-drop. The bacteria are kept in a layer close to the glass, where growth may be studied.

* *Journal of Medical Research.* Vol. VII. March, 1902.

Cover-glass Preparations.—The study of bacteria with the microscope is for the most part done by means of smears made upon thin cover-glasses. It is best to obtain the kind sold by dealers as No. 1, ¾ inch squares.

The cover-glass may be cleaned best by immersion in a mixture of sulphuric acid and bichromate of potassium solution, and afterward washed thoroughly in distilled water, and finally in alcohol. A stock of clean cover-glasses may be kept in a bottle of alcohol.

CLEANING FLUID.

Potassium bichromate	40 grams.
Water	150 c.c.
Dissolve the bichromate of potassium in the water, with heat; allow it to cool; then add slowly and with care sulphuric acid, commercial	230 c.c.

For most purposes it is sufficient to wash the cover-glass in alcohol containing 3 per cent. of hydrochloric acid. It should then be wiped clean with a piece of linen cloth. Whenever it is taken into the fingers it should be held by the edges, never by the flat surfaces. In spreading bacteria upon it and in all subsequent manipulations, as staining, the cover-glass should be handled with the forceps. It can be used very conveniently in the form of forceps known as the Cornet forceps, or in the modification devised by Stewart. Bacteria may be placed upon the cover-glass by allowing the glass to fall upon one of the colonies of bacteria, on a gelatin or agar plate (see page 88), which will adhere to it in part, producing an "impression preparation" (German, *Klatschpreparat*). Such a preparation, after drying in the air, is to be fixed by passing it through the flame three times. (See below.) The forceps with which it is handled should be sterilized in the flame.

Generally bacteria contained in fluids, like sputum, or taken from the surface of a culture, are smeared over the cover-glass by means of the platinum wire or loop, which must be heated to

a red heat before and after the operation. Such preparations are called smear, cover-glass, cover-slip, or film preparations. When the material to be spread is thick or very viscid, a small drop of distilled water must first be placed in the center of the cover-glass so as to dilute it. Beginners generally take too much material on the wire. As thin a smear as possible is made. It

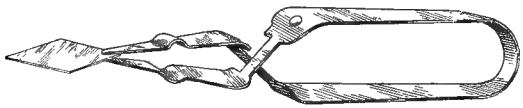

FIG. 7.—CORNET FORCEPS FOR COVER-GLASSES.

is allowed to dry in the air; this should occupy a few seconds. The drying may be hastened by holding the forceps with the cover-glass a long distance above the flame, at a point where the heat would cause no discomfort to the hand. Having dried the preparation, it is to be passed with the smeared surface up three times through the flame of a Bunsen burner or alcohol lamp. The heat of the flame serves to dry the bacteria upon the

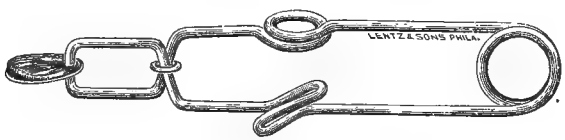

FIG. 8.—STEWART FORCEPS FOR COVER-GLASSES.

cover-glass and fix them permanently in position; it is not sufficient, however, when applied in this manner, to kill all kinds of bacteria, especially those containing spores. After it has been passed through the flame three times the preparation may be stained with a solution of one of the aniline dyes, as described below, and after washing in water and drying may be mounted, face down, in Canada balsam upon a glass slide. It

makes a suitable object to be examined with the oil-immersion objective.

The smear preparation may equally well be made directly upon the glass slide. The fixation in the flame must then occupy a longer time than with the small and thin cover-glass. Such preparations have the advantage that several may be made upon one slide, and that after staining them they may be examined in cedar-oil, with the oil-immersion lens, without the use of the cover-glass and Canada balsam. The forceps of Kirkbride will be found convenient for staining on the slide. Experiments performed in the writer's laboratory have shown that the ordinary method of fixation in the flame, when applied to bacteria spread

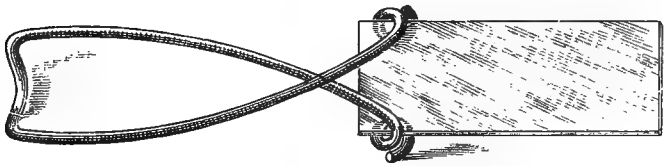

FIG. 9.—KIRKBRIDE FORCEPS FOR HOLDING SLIDES.

upon slides, has little effect on the vitality of many species. The beginner is, therefore, advised to make his preparations on cover-glasses.

When very resistant or dangerous pathogenic bacteria are being handled, after fixation by heat upon the slide or cover-glass, the preparation may, if desired, be immersed in 1–1000 solution of bichloride of mercury long enough to kill the bacteria, without injuring the preparation or interfering with its staining properties.

Staining.—The bacterial cells are devoid of color, and the object of staining them is to give them artificially some color that would make them distinct and easily visible with the microscope. In order that they shall stand out sharply the stain

employed should leave the background unstained. This result is best obtained with aqueous solutions of the aniline dyes. These aniline dyes, so called, are derivatives of coal-tar, but not always of aniline. These dyes are of great importance in bacteriological work, since they are used to make stained preparations. Their number is very large, but only a few are in common use. It is important to have the purest, and those manufactured by Grübler are reliable.

It is simplest to classify the aniline dyes as acid or basic. Eosin, picric acid and acid fuchsin are acid dyes; they tend to stain tissues diffusely. Fuchsin, gentian-violet and methylene-blue are basic dyes; they have an affinity for the nuclei of tissues and for bacteria; they therefore are the dyes used chiefly in bacteriological work. The other kinds may be employed as contrast-stains; another contrast-stain frequently used is Bismarck brown. It is best to keep on hand saturated solutions of the aniline dyes in alcohol, from which watery solutions may be made when needed by adding a few drops of the alcoholic solution to a small dish filled with water. The alcoholic solution is diluted about ten times, so as to make a liquid which is just transparent in a layer about 12 mm. in thickness, after filtering.

Fuchsin and gentian-violet operate rapidly and intensely. Methylene-blue works more slowly and feebly; it is to be preferred where the bacteria occur in thick or viscid substances, like pus, mucus, and milk, and acts more energetically when made slightly alkaline.

Method of Staining Cover-glass Preparations.—(*a*) A smear preparation of bacteria having been made, dried, and passed through the flame three times in the manner above described, and a watery solution of either fuchsin, gentian-violet or methylene-blue having been prepared, the cover-glass is to be dropped into a dish containing the dye, or the dye may be dropped upon the cover-glass held in the forceps.

(*b*) Allow the stain to act for about thirty seconds.

(*c*) Wash in water.

(*d*) Examine with the microscope in water directly or after drying and mounting in Canada balsam.

The rapidity and intensity of staining may be increased by warming the solution slightly. The bacteria will usually appear more distinct if, directly after pouring off the stain, the preparation is rinsed for a few seconds in 1 per cent. solution of acetic acid, and then thoroughly washed in water without materially affecting the stain in the bacteria, which are thus brought out more strongly. The acetic acid solution serves to remove in a measure any color which has been imparted to the background.

Preparations that are mounted at first in water may be made permanent by letting a drop of water fall at the edge of the cover-glass so that it may easily be removed from the slide, then drying and mounting in Canada balsam. Cover-glass preparations which have been stained are examined with oil-immersion objective, employing the plane mirror, having the iris diaphragm open and the condenser close to the lower surface of the glass slide. The purpose is to obtain the most intense illumination possible over a small field. The watery solutions of aniline dyes prepared as above described deteriorate in a short time, and it is best to prepare them freshly each time they are required. A very useful solution, which is permanent, is Löffler's alkaline methylene-blue:

Concentrated alcoholic solution of methylene blue	30 c.c.
Potassium hydrate (caustic potash), 1–10,000 watery solution	100 c.c.

Löffler's methylene-blue is a good stain for general purposes. It is perhaps more in use than any other formula for coloring the diphtheria bacillus.

Aniline-water Staining Solutions.—The intensity with

which aniline dyes operate may be increased by adding aniline oil to the solution:

Aniline oil	5 c.c.
Water	100 c.c.

Mix, shake vigorously, filter; the fluid after filtration should be perfectly clear; add—

Alcohol	10 c.c.
Alcoholic solution of fuchsin (or gentian-violet, or methylene-blue)	11 c.c.

Aniline-water staining solutions do not keep well, and need to be freshly prepared about every two weeks. The applications of the aniline-water stains will be given under separate headings. In general, however, they are employed where a stain of unusual power is required.

Gram's Method.—The advantages of Gram's method are that by using it certain kinds of bacteria may be stained a violet color, while other bacteria are stained feebly or not at all. Cover-glass preparations, having been prepared and fixed in the usual manner (see pages 26 and 27), are stained as follows:

(*a*) Stain in aniline-water gentian-violet solution, from two to five minutes. The intensity of the stain may be increased by warming slightly.

(*b*) Iodine solution, one and one-half minutes:

Iodine	1 gram.
Potassium iodide	2 grams.
Water	300 c.c.

In this solution the preparation becomes nearly black.

(*c*) Wash in alcohol repeatedly; the alcohol becomes stained with clouds of violet coloring matter; the alcohol is used as long as the violet color continues to come away, and until the preparation is decolorized or has only a faint steel-blue color.

(*d*) When desired, the specimens may be stained, by way of contrast, with a watery solution of Bismarck brown or eosin.

(*e*) Wash in water, and examine either in water directly or after drying and mounting in Canada balsam. A modification of this method, sometimes called the Gram-Günther method, differs from the preceding by using a 3 per cent. solution of hydrochloric acid in alcohol for ten seconds to hasten decolorization, washing in pure alcohol before and after the acid alcohol. Decolorization is more intense than by the Gram method; the diphtheria bacillus, which is stained by Gram's method, is decolorized by the Gram-Günther (Kruse).

Bacteria that are stained by Gram's method:

Staphylococcus pyogenes aureus,
Streptococcus pyogenes,
Micrococcus lanceolatus (of pneumonia),
Micrococcus tetragenus,
Bacillus of diphtheria,
Bacillus of tuberculosis,
Bacillus of leprosy,
Bacillus of anthrax,
Bacillus of tetanus,
Bacillus aërogenes capsulatus,
Ray fungus of actinomycosis.

List of bacteria that are not stained by Gram's method:

Gonococcus,
Diplococcus intracellularis meningitidis,
Micrococcus melitensis,
Bacillus of chancroids (Ducrey),
Bacillus of dysentery (Shiga),
Bacillus of typhoid fever,
Bacillus coli communis,
Bacillus pyocyaneus,
Bacillus of influenza,
Bacillus of bubonic plague,
Bacillus of glanders (bacillus mallei),

Bacillus of malignant edema,
Bacillus of Friedländer,
Bacillus proteus,
Spirillum of Asiatic cholera,
Spirillum of relapsing fever.

Staining the Bacillus of Tuberculosis.—Since the tubercle bacillus does not take the ordinary stains readily, a very large number of methods have been proposed for staining it, all of which depend upon the principle that, after adding to solutions of aniline dyes certain substances, like aniline-water, carbolic acid, or solutions of ammonia or soda, the bacillus tuberculosis is stained with great intensity, and gives up its stain with difficulty. Solutions of acids will remove the stain from all parts of the preparation excepting from the tubercle bacilli, which retain the dye, once having acquired it. The rest of the preparation may now be given a different color—contrast-stain.

Bacilli that resist decolorization by acids are called *acid-proof* or acid-fast. The most important are tubercle and leprosy bacilli. There are various other species, however, most of which are less resistant to acids and alcohol than tubercle bacilli. They are discussed in the article on the bacillus tuberculosis in Part IV.

Occasionally spores of other bacteria, micrococci and horny epithelial cells are imperfectly decolorized, but their forms distinguish them from tubercle bacilli. Minute crystalline needles which have a shape like that of bacilli are often encountered in sputum, but their nature will be recognized after a little practice.

The stain for tubercle bacilli is most frequently used for specimens of sputum from cases of suspected pulmonary tuberculosis; it may be applied to other fluids and secretions equally well. It is not reliable, however, when applied to milk, as the oil present in milk interferes with its operation,

and milk and its products quite often contain other acid-proof bacilli. The smegma of the external genitals also frequently contains acid-proof bacilli that are not tubercle bacilli. On this account all fluids and discharges from the genito-urinary tract need to be examined with particular care not to confuse tubercle bacilli with smegma bacilli. (See smegma bacilli in Part II., Chapter IV.)

Patients should be given minute instructions concerning the collection of sputum. The bottle used should be new, wide-mouthed, clean, and kept tightly stoppered with a clean cork. The patient should be cautioned against allowing the expectoration to get on the outside of the bottle. Probably whatever risk is incurred by those who examine sputum comes chiefly from the outside of the bottle having been soiled with sputum containing tubercle bacilli. Often little white particles may be seen floating in the mucous portions of the sputum. These particles should be selected for the investigation, and may be spread in a thin film on the cover-glass with the platinum wire, which is sterilized in the flame before and after using. The selection of the little white particles will be facilitated if the sputum be poured into a clean glass dish, which may be placed on a black surface. A form of porcelain dish is furnished by dealers, the bottom of which is black, and which is convenient, for these manipulations. The smears must be made thin, or the subsequent decolorization, after staining, will not be uniform. It is hardly necessary to observe that the operator must be scrupulously careful not to contaminate the material under examination with any kind of extraneous matter. The cover-glasses and slides which are used should be new, and should have been cleaned with bichromate of potassium and sulphuric acid (see page 25).

When the work is completed, the bottle containing the sputum should be sterilized by steam or boiling.

Many different methods for staining the tubercle bacillus

have been proposed. In most of those now in use the following solution (Ziehl's carbol-fuchsin) is employed—

Fuchsin	1 gram.
Carbolic acid, pure	5 c.c.
Alcohol	10 c.c.
Distilled water	100 c.c.

The method given below is the one recommended.

Method for staining the tubercle bacillus:

(*a*) The cover-glass preparation is made, dried, and fixed by passing through the flame three times (see pages 26 and 27).

(*b*) The cover-glass, held in forceps or in a watch-crystal, is covered with carbol-fuchsin and heated till the stain begins to give off vapor. The stain is allowed to act for five minutes.

(*c*) Wash in water.

(*d*) Wash in alcohol containing 3 per cent. of hydrochloric acid one minute, or longer if necessary to remove the red color.

(*e*) Wash in water.

(*f*) Stain with methylene-blue solution (see page 29) thirty seconds.

(*g*) Wash in water.

(*h*) Examine in water directly, or after drying and mounting in Canada balsam. Tubercle bacilli take a brilliant red color; other bacteria and the nuclei of cells are stained blue.

Gabbett's Method.—This method is very popular and widely used on account of its convenience. It is not as reliable as the one just given.

Gabbett's solution:

Methylene-blue	1 to 2 grams.
25 per cent. watery solution of sulphuric acid	100 c.c.

(*a*) The cover-glass preparation is to be made, dried and fixed by passing through the flame three times.

(*b*) The carbol-fuchsin stain is applied from two to five minutes to the cover-glass, held in forceps or in a watch-crystal; it need not be warmed.

(*c*) Wash in water.

(*d*) Gabbett's solution is applied for one minute.

(*e*) Wash in water. The preparation should have a blue color. It may be examined in water directly or after drying and mounting in Canada balsam.

Gabbett's method has the advantage of decolorizing the preparation and staining the background with methylene-blue at the same time. Tubercle bacilli are colored a brilliant red; most other bacteria and the nuclei of cells are colored blue. The acid-proof bacilli mentioned on page 32 also retain the red stain in most cases, and might be confused with tubercle bacilli.

Of the numerous methods of staining tubercle bacilli, only a few others can be mentioned. Aniline-water fuchsin, aniline-water gentian-violet, or carbol-fuchsin may be used. The intensity of the stain must then be increased by warming the preparation till it steams or boils, then allowing the warm stain to act on the specimens for from three to five minutes; the preparation may also be left in the cold stain over night. Decolorization may be effected with a 25 per cent. solution of sulphuric acid used till the red color disappears, or a 30 per cent. solution of nitric acid, which operates very rapidly. If the red color persists after washing in water, dip in the acid again. After either acid the preparation is to be washed in alcohol until the last trace of the stain has been removed. An excellent decolorizing agent is a 3 per cent. solution of hydrochloric acid in alcohol, used for about a minute. With any of these acid solutions the decolorization can be accomplished more perfectly than with Gabbett's solution, where the operation of the decolorizing agent is masked. The contrast-stain may be omitted entirely if it is desired. A suitable contrast-stain after fuchsin staining is a solution of methylene-blue; after gentian-violet staining, Bismarck brown.

Those who have had experience in staining tubercle bacilli soon discover that the bacilli exhibit some differences in their

resisting power to strong acids. One encounters occasionally bacilli that are perfectly stained side by side with others that are more or less completely decolorized. These facts show the necessity of practice with any method, and of exercising caution and judgment in making a diagnosis where the number of bacilli happens to be scanty. If tubercle bacilli are not found in the first preparation, other preparations should be made. Sometimes a large number of cover-glasses must be examined.

Various expedients have been devised to concentrate tubercle bacilli when only a small number may be present in a sample of sputum. In Biedert's method about 15 c.c. of sputum are mixed with 5 c.c. of distilled water, 4 to 8 drops of sodium hydrate solution are added, and the mixture is boiled. After boiling, add about 15 c.c. of distilled water. The mixture may be set aside in a conical glass for from twenty-four to forty-eight hours when the sediment may be collected, smeared on a cover-glass and stained for tubercle bacilli; or the sediment may be precipitated rapidly by the use of the centrifuge. The sediment will be found to have little adhesive power, and will not stick well to the cover-glass. It is convenient to save some of the original sputum and mix it with the sediment for this purpose.

Staining Bacteria in Tissues.—Pieces of organs about 1 cm. in thickness may be taken. Alcohol is the best agent for preserving them. The fixation will be completed in a few days. It is best to change the alcohol. The amount of the alcohol must be twenty times the bulk of the tissue to be preserved.

Ten parts of the standard 40 per cent. solution of formaldehyde, with 90 parts water make a good mixture for fixation; after twenty-four hours change to alcohol.

Imbedding in Collodion or Celloidin.—From alcohol the pieces of tissue are placed in equal parts of alcohol and ether, twenty-four hours; thin collodion (1½ per cent.), twenty-four hours; thick collodion of a syrupy consistency (6 per cent.),

twenty-four hours. The specimen is laid upon a block of wood or, better, the compressed vegetable fibre called vulcanite, and surrounded by thick collodion, and then placed in 70 per cent. alcohol. The collodion makes a firm mass, surrounding and permeating the tissue, and permits very thin sections to be cut. The soluble cotton sold by dealers in photographers' supplies serves as well as the expensive preparation known as celloidin. To make collodion, dissolve it in equal parts of alcohol and ether. Soluble cotton is also called pyroxylin, and is a kind of gun-cotton.

Imbedding in Paraffin.—(*a*) Pieces of tissue 2 to 3 mm. thick which have already been fixed in alcohol or formaldehyde are to be placed in absolute alcohol for twenty-four hours.

(*b*) In pure xylol one to three hours.

(*c*) In a saturated solution of paraffin in xylol one to three hours.

(*d*) In melted paraffin having a melting-point of 50° C., which requires the use of a water-bath or oven, one to three hours. The xylol must be entirely driven off, and the tissue thoroughly infiltrated.

(*e*) Change to fresh paraffin for one hour.

(*f*) Finally, place the tissue in a small dish or paper box and pour the melted paraffin about it. Harden as quickly as possible with running water. It is important to fix the piece of tissue in a suitable position, if the position is of importance, before pouring in the melted paraffin.

Sections of exquisite thinness may now be cut. The knife need not be wet. Paraffin imbedding is especially desirable when serial sections are to be made.

In order to mount the sections, proceed as follows:

(*a*) Place the sections on water in a porcelain capsule. Warm slightly, when the sections will flatten nicely. Smear the surface of a slide with a very thin layer of Mayer's glycerin-albumen mixture. Dip the slide under the sections; lift them;

and then drain off the water, leaving the sections in their proper positions. Let them dry for some hours in the incubator, and they will be firmly fastened to the slide.

(*b*) Dissolve out the paraffin in one of the numerous solvents (xylol, a few minutes).

(*c*) At this point the xylol should be washed off with absolute alcohol, and

(*d*) The section should be stained.

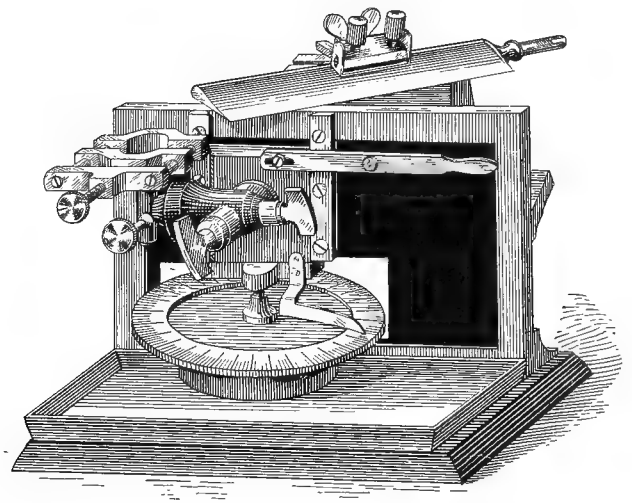

FIG. 10.—SCHANZE MICROTOME.

(*e*) Dehydrate in absolute alcohol.

(*f*) Clear in xylol.

(*g*) Mount in balsam.

GLYCERIN-ALBUMEN MIXTURE (MAYER).

Equal parts of white of egg and glycerin are thoroughly mixed, and then filtered. Add a little gum-camphor to preserve.

Section Cutting.—Cutting is best done with an instrument

called a microtome. The tissues may be imbedded in collodion or paraffin; or when they have been hardened with formaldehyde they may be cut after freezing. Bacteria stain admirably in such frozen sections. For routine work collodion imbedding will be found as convenient a process as any. Paraffin imbedding gives the thinnest sections.

A microtome consists of a heavy, sliding knife-carrier, which moves with great precision on a level, and of a device for elevating the object which is to be cut any desired distance after each excursion of the knife. The thickness of the section will be the distance which the object is elevated. The knife is kept wet with alcohol during the cutting of collodion sections, otherwise it is left dry. The microtome is usually provided with a special form of knife. A razor will serve nearly as well, after having had the lower side ground flat. If a razor is used, a special form of razor-holder must be attached to the microtome to receive the razor. Above all, it is necessary that the knives should be kept in good condition. Only occasionally will they need honing, using a fine water-stone or Belgian hone. Preferably the knife should not be honed directly on the stone itself but on a piece of clean plate glass, on which the stone is first rubbed with water. By this means the entire cutting edge is sharpened in one plane. The movement in honing should be from heel to toe, and toward the cutting edge, always placing the back of the knife next the hone when turning. The knife should be stropped frequently. The leather of the strop should be glued to a strip of wood to make a flat surface. The movement in stropping should be from toe to heel. Sections should be cut to a thickness of not more than 25 μ. Thinner sections (5 to 10 μ) are to be desired.

Staining of Sections.—A watery solution of one of the aniline dyes is used—fuchsin, gentian-violet or methylene-blue—made by adding a few drops of the alcoholic solution to a dish

filled with water. Löffler's solution of methylene-blue serves very well.

By this process most bacteria are stained; also the nuclei of cells; frequently, also, certain granules contained within some cells (German, *Mastzellen*), which may easily be mistaken for bacteria by the inexperienced (basophilic granules).

(*a*) Place the section in the staining solution from two to five minutes.

(*b*) Wash in water.

(*c*) Place in a watery solution of acetic acid, 1 per cent., for one minute.

(*d*) Alcohol, one to two minutes; change to absolute alcohol. Touch the sections to blotting-paper to remove the superfluous alcohol.

(*e*) Xylol until clear; xylol is to be preferred to other clearing agents, like oil of cloves, most of which slowly remove aniline colors. It has the disadvantage of not clearing when the slightest trace of water is present; dehydration in alcohol must, therefore, be complete. The section should be removed from the xylol as soon as it is cleared; otherwise wrinkling occurs.

(*f*) The section is placed upon a glass slide; a drop of Canada balsam is placed upon it and then a cover-glass. The Canada balsam should be dissolved in xylol.

The section is to be manipulated with straight or bent needles. The removal from xylol to the glass slide is managed best with a spatula or section-lifter.

The above statements apply to frozen sections or to sections imbedded in celloidin. Paraffin sections are preferably attached to the slide with glycerin-albumen. The different steps in the process follow in the same order. The stain may be poured on the slide, or the slide may be placed in a large dish full of staining fluid. (See page 37.) Celloidin sections may also be stained on the slide. If the section be well spread and flattened thoroughly with blotting-paper, it will usually adhere

to the slide, and is less likely to wrinkle. It must not be allowed to dry.

Gram's method may be applied to the staining of sections of tissues as well as to smears upon cover-glasses.

(*a*) Place the section in aniline-water gentian-violet, one to five minutes. See the preceding paragraph for the manner of handling sections.

(*b*) Rinse briefly in water.

(*c*) Iodine solution (see page 30), one and one-half minutes.

(*d*) Alcohol, until decolorized to a faint blue-gray.

(*e*) Xylol.

(*f*) Mount on a slide in balsam.

Weigert's Modification of Gram's Method, or Weigert's Stain for Fibrin.—(*a*) Place the section in aniline-water gentian-violet solution, five minutes or more. See page 40 for the manner of handling sections.

(*b*) Wash briefly in water.

(*c*) After placing the section upon a slide, and having straightened it carefully, absorb the water with blotting-paper.

(*d*) Iodine solution (see page 30), one to two minutes.

(*e*) Absorb the iodine solution with blotting-paper.

(*f*) Add aniline oil, removing it from time to time with blotting-paper, and adding fresh aniline oil until the color ceases to come away. (Aniline oil serves in this connection both to decolorize and to dehydrate. It absorbs the water rapidly and efficiently. However, on account of its decolorizing tendency, it must be removed before the specimens can be mounted permanently.)

(*g*) Add xylol; remove it with blotting-paper; and add fresh xylol several times, in order to extract the last trace of aniline oil.

(*h*) Mount in Canada balsam.

This method is more convenient for the staining of sections than the Gram method. The results, however, are essentially

the same as far as the bacteria are concerned; fibrin and hyaline material are stained blue, bacteria violet. It is often impossible to decolorize the nuclei completely without decolorizing the bacteria also. The parts of the nuclei which remain stained often present pictures that resemble bacteria, and which may lead to error if not recognized. Basophilic granules also retain the stain, as do the horny cells of the epidermis. These remarks apply also to Gram's method, except as regards fibrin. Very beautiful preparations can be obtained according to this or the Gram method when the sections have previously been stained in carmine; the nuclei will then be colored red, bacteria violet.

Tubercle bacilli may be *stained in sections* as follows:

(*a*) Use carbol-fuchsin, or aniline-water gentian-violet for one-half to two hours with very gentle warming, or over night without warming. See page 40 for the manner of handling sections.

(*b*) Wash in water.

(*c*) Decolorize with some one of the decolorizing agents mentioned in connection with the staining of tubercle bacilli in cover-glass preparations, preferably 3 per cent. hydrochloric acid alcohol. Decolorization must be continued until the red color has disappeared, which requires one-half to several minutes.

(*d*) Wash in alcohol.

(*e*) Wash in water.

(*f*) Use hematoxylin as a contrast-stain for fuchsin preparations, and carmine for gentian-violet preparations. In the latter case it is better to stain with carmine before staining the bacilli. The carmine is not affected by the subsequent treatment.

(*g*) Wash in water.

(*h*) Alcohol.

(*i*) Xylol.

(*j*) Balsam.

Nuclear stains, which may be used as contrast-stains for sections:

DELAFIELD'S HEMATOXYLIN.

Hematoxylin crystals	4 grams.
Alcohol	25 c.c.
Ammonia alum	50 grams.
Water	400 c.c.
Glycerin	100 c.c.
Methyl-alcohol	100 c.c.

Dissolve the hematoxylin in the alcohol, and the ammonia alum in the water. Mix the two solutions. Let the mixture stand four or five days uncovered; it should have become a deep purple. Filter and add the glycerin and the methyl-alcohol. After it has become dark enough, filter again. Keep it a month or longer before using; the solution improves with age. At the time of using, filter and dilute with water as desired.

LITHIUM-CARMINE (ORTH).

Carmine	2.5 grams.
Saturated watery solution of lithium carbonate	100.0 c.c.

Add a few crystals of thymol. The carmine dissolves readily in the lithium carbonate solution. Filter the stain at the time of using. Sections are to be left in the stain five to twenty minutes.

Sections stained in carmine are placed directly in acid alcohol (1 part hydrochloric acid, 100 parts 70 per cent. alcohol) for five to ten minutes. They acquire a brilliant scarlet color. When used as a contrast-stain for tissues containing bacteria, it is best to use it before staining the bacteria, which might be decolorized by the acid alcohol.

Staining of Blood-films.—The method of *Wright* is the one recommended. It is applicable to bacteria and to the parasite of malaria, and is useful as a general stain for blood.

Films of blood are prepared as directed in Chapter VII., Part I., and are allowed to dry.

(*a*) The stain is poured over the surface of the preparation till it covers it. This serves to fix the film of blood. It is allowed to remain for one minute.

(*b*) Add distilled water, drop by drop, till a reddish tint appears at the edges and a metallic scum forms on the surface. About six drops are needed for a three-fourths inch cover-glass. The real staining of the preparation now takes place, and requires two or three minutes.

(*c*) Wash in distilled water till the thin parts of the preparation have a yellowish or pinkish tint, which requires one to three minutes.

(*d*) Dry with blotting-paper and mount in Canada balsam.

Bacteria, malarial parasites, and cell-nuclei are stained blue, red blood-corpuscles are orange-pink, while the specific granules of the leucocytes (neutrophilic, etc.) appear in various tints from red to dark blue. The chromatin of the malarial parasite takes a lilac to red color. The blood-plates have a bluish or purplish color and must not be confused with malarial parasites.

The staining fluid is prepared as follows: To 100 c.c. of a 1 per cent. solution of sodium bicarbonate in water add 1 gram of methylene-blue. Place in the steam sterilizer at 100° C. for one hour. When cool add one-tenth per cent. watery solution of eosin (Grübler, yellowish, soluble in water) until the mixture loses its blue color, becomes purple, and a metallic scum forms on the surface. About 500 c.c. of the eosin solution are needed. Collect the precipitate on a filter; let it dry; make a saturated solution of the precipitate in methyl-alcohol; filter. To the quantity obtained add one-fourth as much methyl-alcohol, so that the solution may not be completely saturated. The purpose of the above procedures is to modify the methylene-blue so that other staining elements are developed in it (polychromism). The modified methylene-blue solution is then combined with eosin. For full details see Wright. *Journal of Medical Research.* Vol. VII. 1902.

Staining of Spores.—The method is applicable to cover-

glass preparations which may be prepared in the usual way from material supposed to contain spores.

(*a*) After drying the smear on the cover-glass, and fixation with heat by passing through the flame three times, use as a stain aniline-water fuchsin.

(*b*) Heat until the preparation begins to boil; remove for a minute; heat again, and again remove; repeat this process six times.

(*c*) Wash in a weak solution of acetic or hydrochloric acid for a few seconds to a minute. Some spores are quickly decolorized by 1 per cent. acetic acid; others may keep the stain when subjected to 3 per cent. hydrochloric acid alcohol for a minute.

(*d*) Wash in water.

(*e*) Stain with watery solution of methylene-blue half a minute.

(*f*) Wash.

(*g*) Dry.

(*h*) Balsam.

The spores are intensely stained by the fuchsin. The stain is removed from everything except the spores by the acid. The methylene-blue solution stains the bodies of the bacteria, the spores remaining brilliant red. There are various other methods for staining spores, but this procedure gives good results. The principle is the same as in staining the tubercle bacillus, except that more pains are needed to impregnate spores with the dye.

Staining of Capsules.—The capsules which many bacteria possess appear to be made of gelatinous substance, which is difficult to stain.

Method of Welch.—(*a*) Cover-glass preparations are made in the usual manner. Pour glacial acetic acid over the film.

(*b*) After a few seconds, replace with aniline-water gentian-violet, without washing in water. Change the stain several

times to remove all the acetic acid. Allow it to act three or four minutes.

(*c*) Wash and examine in salt solution, 0.8 to 2.0 per cent.

Bacteria are deeply stained, while their capsules are pale violet. This method has been recommended for staining the capsule of the pneumococcus.

Methods of Hiss.—1. (*a*) Cover-glass preparations are made in the usual manner, and fixed in the flame.

(*b*) Stain for a few seconds in a half-saturated watery solution of gentian-violet.

(*c*) Wash in solution of potassium carbonate in water.

(*d*) Mount and study in the same.

2. (*a*) Cover-glass preparations are made and fixed in the ordinary way.

(*b*) Use the following stain, heated till it steams:

Saturated alcoholic solution of gentian-violet or fuchsin	5 c.c.
Distilled water	95 c.c.

(*c*) Wash in 20 per cent. solution of cupric sulphate.

(*d*) Dry and mount in Canada balsam.

The methods of Hiss are recommended to be used for bacteria that have been cultivated on media containing blood-serum. They have shown that many streptococci have capsules. The writer has had good success from the latter method, with preparations of the pneumococcus from animal tissues.

Staining of Flagella.—Flagella are among the most difficult of all objects to stain. The best-known method is that of *Löffler*. It is important to use young cultures, preferably on agar.

(*a*) A small portion of the culture is mixed on a cover-glass with a drop of water. The preparations must be *exceedingly thin*. The mixing must be done with care in order not to break off the delicate flagella. The cover-glass must be perfectly clean (see page 25).

(*b*) After drying, fixation is effected by passing through the flame three times.

(*c*) The essential point in this method is the use of a mordant as follows:

Tannic acid, 20 per cent. solution	10 c.c.
Saturated solution of ferrous sulphate	5 c.c.
Saturated alcoholic solution of fuchsin	1 c.c.

This solution is filtered and a few drops are placed on the cover-glass, or the cover-glass is placed, face down, in a dish containing the stain; it is then left for one to five minutes, warming slightly.

(*d*) Wash in water.

(*e*) Stain with aniline-water fuchsin, or carbol-fuchsin.

(*f*) Wash in water.

(*g*) Dry.

(*h*) Mount in Canada balsam.

(According to Löffler, certain bacteria require the addition of an acid solution, and certain others an alkaline solution, but many observers consider this unnecessary.)

Another and very valuable method is that of *Van Ermengem.*

(*a*) Make and fix cover-glass preparations as in the preceding method.

(*b*) Use the following mordant for one-half hour at room-temperature or for five minutes at 50° to 60° C.

Osmic acid, 2 per cent. solution	1
Tannic acid, 10 to 25 per cent. solution	2

(*c*) Wash carefully in distilled water and then in alcohol.

(*d*) Place for a few seconds in a 0.25 to 0.50 per cent. solution of nitrate of silver—"the sensitizing bath."

(*e*) Without washing transfer to the "reducing and reinforcing bath":

Gallic acid	5 grams.
Tannic acid	3 grams.
Fused potassium acetate	10 grams.
Distilled water	350 c.c.

(*f*) After a few seconds, replace the preparation in the nitrate of silver solution, in which it is kept constantly moving, till the solution begins to acquire a brown or black color.

Some recommend leaving the preparation in the nitrate of silver solution for two minutes in the first place, and in the reducing bath for two minutes, without using the nitrate of silver solution a second time.

(*g*) Finally wash in distilled water, dry, mount in Canada balsam. It is difficult to avoid the formation of precipitates; otherwise the results of this method are usually good.

CHAPTER II.

STERILIZATION.

By sterilization is meant the killing of all microörganisms found on or in any body or substance. It is possible to sterilize objects by the use of bichloride of mercury (corrosive sublimate), carbolic acid and other chemical agents, but their value in practice is often overrated. Sterilization is usually accomplished by heat. The most effective sterilization is that done by steam and by boiling; they are not, however, suitable for all kinds of material.

The naked flame of the Bunsen burner or the alcohol lamp is used largely for the sterilization of small articles. It is evident that no more efficient way of sterilization could be devised than by burning objects, or subjecting them to a red heat. The uses of this method will at once suggest themselves; for instance, surgical dressings that have become soiled with discharges and similar materials can be most easily disposed of by simply burning them up. In laboratory work the flame is constantly employed for the sterilization of the platinum wire, forceps, pipettes and cover-glasses; occasionally test-tubes are sterilized in this manner.

Hot-air Sterilization.—Hot air, at a temperature of 150° C., or higher, maintained for an hour, is very valuable for some materials, although less effective than steam. It has been found that the spores of certain bacteria are not killed even by exposure to this temperature, but it is sufficient for ordinary conditions. Hot-air sterilization is employed for glassware such as Petri dishes, flasks and test-tubes. Flasks and test-tubes are generally plugged with raw cotton. The heating should not be

allowed to go to the extent of scorching the cotton; but a faint light browning of the outside is permissible, and is a guarantee that the sterilization is effectual. Glassware should be placed within the sterilizer when it is cold, and after heating should be allowed to cool gradually in order to avoid breaking. Hot-air sterilization is never used for culture-media.

The apparatus used for hot-air sterilization consists of a box

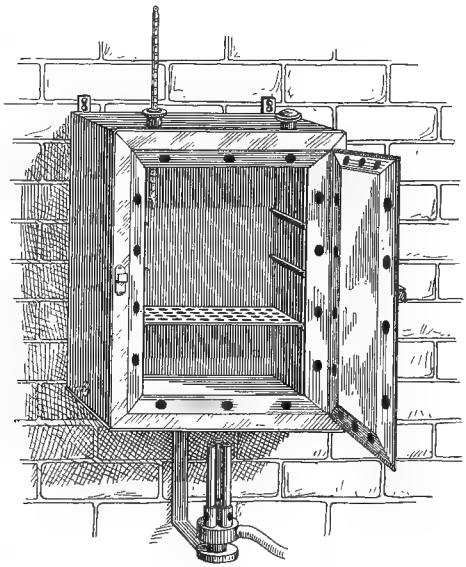

FIG. 11.—HOT-AIR STERILIZER.

made of sheet-iron, the walls being double, with an air-space between them. On one side is a door. There are openings at the top to secure the circulation of air in the air-chamber. A thermometer passes from the top into the interior of the sterilizer so that one may read off the temperature that is being attained. The sterilizer should be placed so that there will be no danger of its setting fire to inflammable articles, as the heat

may occasionally become very intense. It is well, if possible, to have it fastened to a brick wall.

Boiling.—Boiling is an efficient method of sterilization. It is often used for instruments. In laboratory work steam is generally substituted for it.

Steam Sterilization.—Steam sterilization is the most generally used of all forms of sterilization and is the most effective. It is employed for perishable bodies which would be injured by dry air sterilization or by chemical germicides; for example, it is used for surgical instruments and for culture-media; in laboratory work, especially for culture-media. It has been found that there are some forms of bacteria which, in the resting or spore stage, can resist even the action of steam for several hours. Such prolonged exposure to steam would be very injurious to culture-media, which are more or less unstable organic substances. What is called *fractional*, *intermittent* or *discontinuous* sterilization is used for such materials. By that plan the medium is sterilized with steam for fifteen minutes on each of three consecutive days. The object of intermittent sterilization as explained by Tyndall, who proposed it, is this: The culture-medium may be supposed to contain fully developed bacteria, and also bacteria in the spore or resting stage. The first sterilization of fifteen minutes will probably be sufficient to destroy all the fully developed bacteria; during the twenty-four hours between the first and second sterilization all of the spores which have survived the first sterilization may be expected to have become fully developed into bacteria which can be destroyed by the second sterilization; the third sterilization is directed against any spore forms which may possibly have survived the second sterilization.

Although the spore forms which are so extremely resistant are non-pathogenic, as for example spores of the hay bacillus and of the potato bacillus, they nevertheless are capable of ruining the culture-media with which one works.

It has been shown by Theobald Smith that the discontinuous method cannot be relied upon to sterilize fluids in shallow layers that are freely exposed to the air. For if the spores of anaërobic bacteria happen to be present in such fluids, they will not develop into the adult form between the applications of heat, under aërobic conditions.

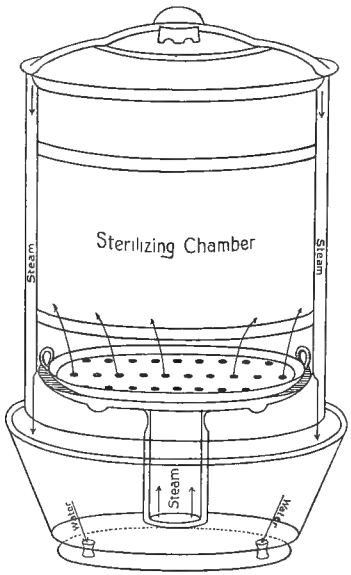

FIG 12.—DIAGRAM OF THE ARNOLD STEAM STERILIZER.

The form of sterilizer most widely used in the United States is that which is known as the Arnold Steam Sterilizer.

The Arnold sterilizer consists of a cylinder of tin or copper with a cover, which is enclosed in a movable, cylindrical outer cover or hood. The inner cylinder has an opening in the bottom through which steam may enter, the steam coming from a small chamber underneath with a copper bottom to which the flame is applied. The peculiarity of this form of sterilizer

consists in the fact that the steam which escapes from the sterilizing chamber condenses beneath the outer cover or hood and falls back upon the pan over the chamber in which the steam is generated. The bottom of this pan is perforated with three small holes, which allow the water of condensation to return into the chamber where the steam is generated. The sterilizer, therefore, to a certain extent, supplies itself with

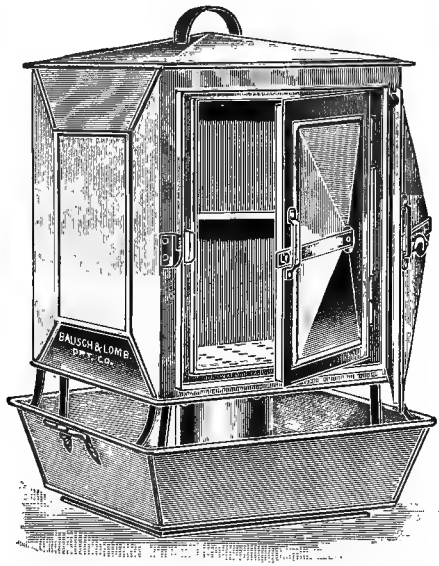

FIG. 13.—STEAM STERILIZER, MASSACHUSETTS BOARD OF HEALTH.

water, although not by any means perfectly. It is, however, less likely to boil dry than other forms of sterilizers, and it has the advantage of being reasonably cheap and quite effective. The space enclosed by the hood also serves as a steam-jacket and helps to overcome fluctuations in temperature. A great improvement upon the ordinary Arnold sterilizer is the modification of it devised by the Massachusetts Board of Health.

In the use of this, or any form of steam sterilizer, the time is

noted from the period when boiling is brisk and it is evident that the sterilizing chamber is filled with hot steam; or, what is better, when the thermometer registers 100° C., if the sterilizer be provided with a thermometer. With a large Arnold sterilizer a temperature of 100° C. may not be reached until it has been heated with a rose-burner for twenty to thirty-five minutes. When bulky articles or large amounts of material

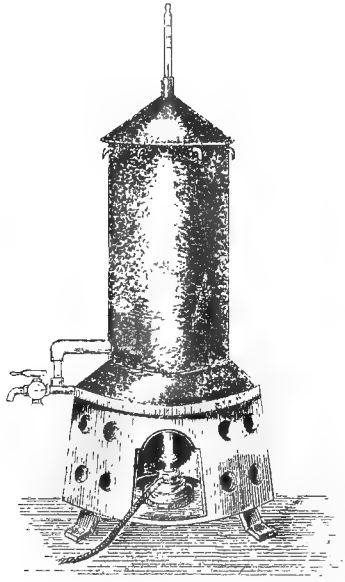

FIG. 14.—KOCH'S STEAM STERILIZER.

are to be sterilized allowance must be made for the time necessary to bring the temperature in the middle of the mass to 100° C.

The sterilizer invented by Koch is still largely in use. It is a tall, cylindrical, tin vessel covered with asbestos or felt. The lower portion is filled with water; on the side is a water-gauge indicating the height of the water, in order that one may ob-

serve when there is danger of the sterilizer boiling dry. Over the top there is a tight-fitting cover. The steam is generated by a Bunsen burner standing underneath. A perforated shelf placed some distance above the surface of the water is for the reception of the tubes and flasks that are to be sterilized.

The *sterilization* of *blood-serum* sometimes has to be performed in a specially devised sterilizer, when a clear, fluid medium is desired. In this case the serum is heated for an hour on each of six consecutive days to a temperature of only 58° C. To obtain a transparent but solid medium the serum is kept at a temperature of 75° C. for an hour on each of four consecutive days. The process must be conducted carefully to avoid clouding of the serum.

Pasteurization.—The name pasteurization has been applied to the partial sterilization of substances at a comparatively low temperature. It is employed particularly for milk. The temperature used (70° to 75° C. for 20 to 30 minutes) is sufficient to destroy all ordinary pathogenic bacteria; for example, the bacilli of tuberculosis and typhoid fever. Furthermore, the great majority of the saprophytic bacteria are destroyed, and milk which has been pasteurized will remain unchanged for several days, if kept cool. Its application is principally in the feeding of infants when ordinary milk has been found to produce undesirable results. Freeman* has invented a pail of special form for the pasteurization of milk in bottles. This pail is filled with hot water and the bottles are placed in it; it has been found to keep up a temperature of about 75° C.

The Autoclave.—The autoclave is an apparatus designed for sterilization by steam under pressure. It was invented in France, but is now used extensively in all parts of the world. Steam generated at the ordinary atmospheric pressure is much less destructive to bacteria, and especially to their spores, than steam in the autoclave at a pressure of an additional one-half to one atmosphere; the steam then reaches a temperature of

* *Medical Record.* July 2, 1892, and August 4, 1894. This pail is sold by James T. Dougherty, 411 West Fifty-ninth Street, New York.

about 112° to 120° C. Under these conditions culture-media may be sufficiently sterilized in the autoclave in fifteen minutes, and at a single sterilization. The autoclave consists of a metal cylinder with a movable top, which is fastened down tightly during sterilization. It is furnished with a thermometer, a pressure gauge, and a safety-valve which allows the steam to escape if too high a pressure is attained. Heat is furnished by

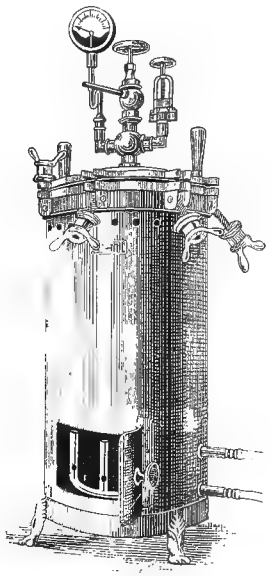

FIG. 15.—AUTOCLAVE.

a gas-burner underneath. The lower part of the cylinder contains water. The objects to be sterilized are supported above this water on a perforated bottom or shelf.

It is necessary to follow certain precautions in the use of the autoclave, especially during cooling. The apparatus must not be opened while the steam contained within it is still under pressure, as there may be a sudden evolution of

steam upon the removal of the pressure which may blow the media out of their tubes and flasks. The apparatus must, therefore, be kept closed until the gauge shows that the atmospheric pressure is as great as the pressure within, or, what is equivalent, until the temperature has fallen to 100° C. Gelatin, especially, may be damaged by sterilization with the autoclave, if it be heated too long or to too high a temperature. Media containing sugar should not be sterilized in the autoclave (see page 62).

Sterilization by Filtration.—Ordinary filters are useless for this purpose, but the tubes or bougies of unglazed porcelain devised by Pasteur and Chamberland are effective when properly employed. They are made in several different grades of porosity. In the Berkefeld filter bougies made of infusorial earth are used, and the pores in this are larger than those of the Pasteur filter. The coarser of these filters permit the passage of very small bacteria. Bacteria of average size, like bacillus coli communis, may grow through the pores in the walls of both the Berkefeld and Pasteur filters if sufficient nutrient material is present to permit of their multiplication.*

FIG. 16.—KITASATO FILTER.

Filters of these kinds are widely used for water, and will be spoken of in connection with the chapter on water. Similar tubes are employed for the filtration of certain organic nutrient media whose ingredients would be damaged by sterilization with heat, chiefly extracts of organs, such as the thymus gland. The soluble "toxins" of bacteria may be obtained by filtration of fluid-cultures through such tubes, which remove

* Wherry. *Journal of Medical Research.* Vol. VIII. 1902.

the bacteria (Fig. 16). These fluids usually filter very slowly, and filtration has to be assisted by some form of vacuum-pump; usually the filter-pump, which is used in connection with a stream of running water, is employed. Compressed air or carbonic acid may be used to assist in forcing fluids through the filter. The filter bougies, the flasks and all parts of the apparatus must, of course, be sterilized by heat before and after using.

CHAPTER III.

CULTURE-MEDIA.

Culture-media are substances in which bacteria are artificially cultivated. The number of such substances is very large, different materials being suited to different purposes and to different kinds of bacteria. The most important ones are nutrient bouillon or beef-tea, nutrient gelatin, and nutrient agar-agar. The two last have a jelly-like consistency, owing to the addition of a gelatinizing substance, but otherwise are of the same composition as bouillon.

Nutrient Bouillon.

Beef-extract (such as Liebig's)	3 grams.
Peptone, pure (Witte's)*	10 grams.
Sodium chloride (common salt)	5 grams.
Water	1 liter.

The solid ingredients are dissolved in water, and the mixture is boiled for a few minutes. It is made neutral or very faintly alkaline by the addition of a solution of sodium hydroxide, drop by drop, the reaction being tested at intervals with litmus-paper. As soon as the proper reaction is reached, it is filtered through filter-paper. The filter-paper should be folded and creased as is done by pharmacists; it is in the usual manner placed in a glass funnel, and should be moistened with water before using. After filtration the medium is to be placed in properly plugged tubes or flasks, and is to be sterilized once in the autoclave, or

* Commercial "peptones" are mixtures of albumose and a small amount of peptone.

in the steam sterilizer for fifteen minutes or longer on each of three consecutive days. When precipitates form, they are usually caused by a too alkaline reaction. That may be corrected by the addition of a little weak hydrochloric acid, drop by drop, testing frequently with litmus-paper.

A more accurate way of obtaining the proper reaction is Schultz's method. Take of the bouillon 10 c.c.; add a few drops of phenolphthalein* (alcoholic solution, ½ per cent.); with a burette add, drop by drop, a solution of caustic soda, 0.4 per cent., until a faint red color appears, which indicates the beginning of the alkaline reaction. This procedure is followed with three samples. The amount of soda solution required in each case is noted and the average taken. If now, on the average, for each 10 c.c. of bouillon 1 c.c. of soda solution needs to be added, for 1000 c.c. of bouillon 100 c.c. of the soda solution must be added; only, instead of adding a weak soda solution, one-tenth as much is taken of a solution ten times as strong.

Another method of making bouillon is to use, instead of beef-extract, 500 grams (one pound) of finely chopped, lean beef, which is placed in one liter of water and kept on ice for twenty-four hours. It is strained, thoroughly cooked to coagulate the albumen in it, filtered and a liter of fluid obtained, adding water if necessary. The peptone and salt are then added and the medium heated to dissolve them. It is then neutralized, filtered and sterilized. Although bouillon made with solid beef-extract is convenient and serviceable for most purposes, it is advisable to use meat when the bouillon is to be employed for the development of bacterial toxins. Meat should also be used in the preparation of either bouillon, gelatin or agar-agar when new species of bacteria are being studied for publication.

In both of these cases the recommendations of the American Public Health Association should be followed.†

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* In neutralizing an acid culture-medium it has been found that when the medium appears to be neutral or slightly alkaline to litmus, it may still be acid if phenolphthalein be employed as an indicator. Fuller. *Journal American Public Health Association.* 1895.

† See the Report of the Committee of the American Public Health Association entitled Procedures Recommended for the Study of Bacteria. 1898. Rumford Press, Concord, N. H.

These also advise that media be neutralized by *titration.*

The following solutions are required: ½ per cent. phenolphthalein in 50 per cent. alcohol, normal * ($\frac{N}{1}$) and twentieth normal ($\frac{N}{20}$) solutions of sodium hydroxide and of hydrochloric acid.

To 5 c.c. of bouillon in a porcelain evaporating dish add 45 c.c. of distilled water; boil three minutes; add 1 c.c. of phenolphthalein solution, and proceed with the titration while still hot. As the reaction will usually be found acid, add from a burette $\frac{N}{20}$ sodium hydroxide solution, stirring constantly, until a decided pink color develops in the entire solution. The color reaction indicates the more or less arbitrarily adopted neutral point. Repeat this procedure with three different portions of bouillon, and determine the average amount of $\frac{N}{20}$ sodium hydroxide required. It is now possible to calculate the amount of $\frac{N}{1}$ sodium hydroxide needed to neutralize the whole quantity of bouillon. This should be added. The bouillon should then be boiled for ten minutes, and again titrated. It will usually be found acid. The deficiency should be corrected by adding the necessary amount of $\frac{N}{1}$ sodium hydroxide. It should be boiled again, and again titrated, and any deficiency made good.

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* A normal solution of any substance contains, in a liter, as many grams of the substance as there are units in its molecular weight, in case it contains a single atom of replaceable hydrogen. If it has two atoms of replaceable hydrogen the number of grams used equals the molecular weight divided by two; and so on. Thus the molecular weight of sodium hydroxide is 40, and its normal solution contains 40 grams of sodium hydroxide in a liter. It is not expedient to prepare normal solutions of sodium hydroxide by weight. For convenience, crystallized oxalic acid is used as a starting point in making normal solutions. Its molecular weight, including a molecule of water of crystallization, is 126. As it is a dibasic acid (having two atoms of replaceable hydrogen), half of this weight, or 63 grams, per liter, is taken. Any $\frac{N}{1}$ acid solution will exactly neutralize an equal volume of any $\frac{N}{1}$ alkaline solution. To make $\frac{N}{1}$ sodium hydroxide solution, add about 41 grams of pure caustic soda to a liter of distilled water. Find the amount of this solution needed to exactly neutralize 1 c.c. of $\frac{N}{1}$ solution of oxalic acid; this amount contains the quantity of sodium hydroxide which should be present in 1 c.c. of a normal solution. It is now possible to calculate the amount of distilled water to be added in order that 1 c.c. of the sodium hydroxide solution may neutralize 1 c.c. of the $\frac{N}{1}$ solution of oxalic acid. With an $\frac{N}{1}$ solution of sodium hydroxide as a standard, an $\frac{N}{1}$ solution of hydrochloric acid may be prepared. Twentieth normal solutions have one-twentieth the strength of normal solutions.

It is rarely necessary to repeat the process, except to determine that the neutral point has been reached. After neutralizing it is boiled thirty minutes and filtered. Enough $\frac{N}{1}$ hydrochloric acid or sodium hydroxide is added to give the degree of acidity or alkalinity desired. It is then sterilized.

An acid reaction may be denoted by +, an alkaline by —. The degree of acidity or alkalinity may be indicated by the amount of $\frac{N}{1}$ solution required to render the medium neutral to phenolphthalein, thus + 1.5 signifies that a medium is acid, and requires 1.5 per cent. of $\frac{N}{1}$ sodium hydroxide to neutralize it.

A reaction of + 1.5 is recommended as the optimum. There is much disagreement as to what reaction is most favorable for the growth of the majority of species of bacteria. In any case the degree of reaction should be noted in descriptions.

Bouillon may be modified by the addition to it of other substances, the most important of which are glycerine (6 per cent.) and sugars,—as dextrose,* saccharose or lactose (1 per cent.). It is better to sterilize media containing sugars in the steam sterilizer by the fractional method than in the autoclave, where decomposition of the sugars may occur.

Dextrose-free Bouillon.—Ordinary bouillon often contains some muscle-sugar, which is objectionable if fermentation tests with lactose or saccharose are to be made. Muscle sugar must also be removed from the beef-juice in preparing diphtheria for the production of antitoxine. To secure bouillon free of sugar, beef-infusion is prepared from meat, and is inoculated in the evening with a quantity of bacillus coli communis, and kept in the incubator. Early next morning it is boiled, filtered, peptone and salt added, and the bouillon is prepared as usual.†

Nutrient Gelatin.

Beef-extract	3 grams.
Peptone	10 grams.
Sodium chloride	5 grams.
Gelatin (best gold label)	100 grams.
Water	1 liter.

Dissolve the ingredients in the water, stirring actively to prevent burning at the bottom. It is best to conduct the opera-

* Dextrose is the principal ingredient of commercial grape-sugar or glucose and should be obtained in a pure condition.

† See Theobald Smith. *Journal of Experimental Medicine.* Vol. II., p. 546.

tions in granite or enamel-ware vessels over a large Bunsen or rose-burner. Neutralize with sodium hydroxide solution (see page 59). Litmus-paper or titration may be used for testing. The reaction at the beginning will usually be found to be quite acid. Allow the mixture to cool until below 60° C., and add the whites of one or two eggs which have been beaten up with a little water; stir in thoroughly. Heat the mixture to the boiling-point; stir at the bottom to prevent burning and at the same time avoid as far as possible breaking the coagulum of egg-albumen which forms at the surface. Boil for ten minutes. Filter while hot. The filtration may be done through folded filter-paper which has been moistened. It is well to fasten a piece of coarse cheese-cloth over the top of the funnel to catch the large particles of coagulated albumen. Place in suitable tubes or flasks plugged with cotton, and sterilize once in the autoclave, or, preferably, in the steam sterilizer for fifteen minutes on each of three consecutive days. Gelatin is injured by too prolonged boiling and loses its solidifying qualities. The remarks on pages 60 to 62 with regard to the use of beef and the titration method for the preparation of bouillon apply equally to gelatin.

Instead of filter-paper, some prefer to filter through several layers of absorbent cotton placed inside of the moistened glass funnel, the top of which is covered with coarse cheese-cloth. This expedient answers very well.

If the product appears cloudy after it has been sterilized, it may be that the egg-albumen was incompletely coagulated in the first place or that the reaction has been made too alkaline. In any case it will be desirable to melt it and filter a second time, correcting the reaction with hydrochloric acid if necessary. It may be well to stir in another egg to entangle the opaque particles; then to boil a second time and filter.

The medium is sometimes modified by adding to it other substances, as sugar, glycerin, etc. The solidifying property

of the gelatin must be carefully guarded, and too much boiling is to be avoided. Certain bacteria, it will be found, have the property of causing gelatin to become permanently liquid: this is called *liquefaction* or *peptonization*. Gelatin melts at about 25° C. and solidifies at about 10° C. It cannot be used in the incubator, where it would melt at the temperature of 38° C. In hot weather it may be necessary to use 150 grams of dry gelatin to the liter. Nutrient gelatin is usually spoken of simply as "gelatin."

Nutrient Agar-agar.—*Agar-agar* (French, *gêlose*) is a kind of vegetable gelatin which comes from the southern and eastern coast of Asia. It melts with much greater difficulty than gelatin, and remains solid at much higher temperatures. In this respect it behaves very peculiarly, since it will not melt unless it is heated to about 80° C.; but after it is once melted it remains fluid at 40° C., or over. After it solidifies it has to be heated up to about 80° C. before it will melt again.

The medium is not quite transparent. The finished medium is commonly called "agar."

Beef-extract	3 grams.
Peptone	10 grams.
Sodium chloride	5 grams.
Agar	10 grams.
Water	1 liter.

The dry agar, cut fine, is to be dissolved in water over a flame. It should be boiled for from one-half hour to two hours, skimming off the scum which forms on the surface from time to time. The beef-extract, peptone and sodium chloride are dissolved in a liter of water, boiled and neutralized. Add the agar now in solution in a small quantity of water. The reaction of the agar alone is faintly alkaline. Mix thoroughly; the bulk of the mixture is a little more than a liter, and should be reduced to a liter by the subsequent boiling. Cool to about 60° C.; stir in the whites of one or two eggs and

boil thoroughly. Avoid breaking the coagulum of egg which is designed to entangle the solid particles that make the medium cloudy; stir at the bottom, however, to prevent burning. Filter while hot, using filter-paper or absorbent cotton covered with cheese-cloth. The hot water funnel originally devised for the filtration of agar is not necessary. If filtration is slow, the funnel and flask may be placed inside of the steam sterilizer and kept heated during filtration. The medium is collected in suitable flasks or tubes plugged with cotton, and sterilized once in the autoclave or in the ordinary steam sterilizer for fifteen minutes on each of three consecutive days. As agar is frequently used for smear-cultures where a slanted medium is desired, some of the tubes may be allowed to cool in a slanting position. It is not well to keep on hand many tubes which have been slanted, as the medium dries more rapidly. Agar is seldom liquefied by bacteria, though a few bacteria possess the power of doing this. Its solidifying qualities are impaired somewhat if the reaction be acid.

The remarks on pages 60 to 62 with regard to the use of beef and the titration method for the preparation of bouillon apply equally to agar-agar.

Glycerin-agar is used extensively. It is agar, made as above directed, to which 6 per cent. of glycerin is added before sterilization. It is very useful in cultivating the bacilli of tuberculosis and diphtheria.

Sugar-agar.—Before sterilizing, 1 per cent. of either dextrose, lactose, saccharose or other sugars may be added to agar. With media containing sugar, *litmus* forms a useful indicator of the production of acid. Enough tincture of litmus is used to give the medium a blue color before sterilization; the litmus is somewhat unstable and prone to change its color during sterilization. *Neutral red* may also be added in the same manner; its color is said to be changed by certain bacteria and not by others (see bacillus of typhoid fever and bacillus coli

communis, Part IV.). To 1 liter of nutrient agar, add 1 gram of dextrose and 0.5 gram of neutral red. Sterilize as usual.

Potato.—The potatoes are washed, a slice is removed from each end, and with an apple-corer or cork-borer a cylinder is cut out. This cylinder is divided diagonally into two pieces. The pieces are washed in running water for several hours. They are placed in test-tubes containing a little water to keep the potato moist, and are supported from the bottom on a piece of glass tubing about 1 to 2 cm. in length (or on cotton, or in a specially devised form of tube with a constriction at the bottom). The tubes are plugged, and sterilized as with other media. Sterilization, however, must be thorough on account of the danger of contamination with the extremely resistant spores of the potato bacillus. Potato is best when freshly prepared; it is likely to become dry and discolored with keeping. It is a very useful medium; certain growths on it, like those of the bacillus of typhoid fever or of glanders, and those of chromogenic bacteria, are very characteristic.*

FIG. 17.—TUBE CONTAINING POTATO.

Milk.—Milk fresh as possible is placed in a covered jar, sterilized for fifteen minutes, and then kept on ice for twenty-four hours. At the end of that time the middle portion is removed by means of a siphon. The upper and lower layers must not be taken; the upper part contains cream, and the lower part particles of dirt, both of which are to be avoided.

* Bolton. *The Medical News.* Vol. I., 1887. P. 318. A Method of Preparing Potatoes for Bacterial Cultures. Roux. De la Culture sur Pomme de Terre. *Annales de l'Institut Pasteur.* T. II., 1888. P. 28. Globig. Ueber einen Kartoffel-Bacillus mit ungewöhnlich widerstandsfähigen Sporen. *Zeitschrift für Hygiene.* P. 322. 1888.

About 7 to 10 c.c. are to be run into each test-tube. The tube is plugged with cotton, and sterilized as usual. When milk is contaminated with spores of the hay or potato bacillus it is sometimes very difficult to sterilize, a fact of much importance in connection with the feeding of children, where the fractional method of sterilization and the use of the autoclave are impracticable.

The coagulation of milk, which is accomplished by certain bacteria, is a very valuable differential point. A little litmus tincture may be added to the tubes of milk before sterilizing, until they acquire a blue color, to indicate whether or not acids are formed by the bacteria which are afterward cultivated in the milk.

Dunham's Peptone Solution.

Peptone	10 grams.
Sodium chloride	5 grams.
Water	1 liter.

Boil, filter, sterilize in the usual manner.

Dunham's solution is valuable to test the development of indol by bacteria (see Part II., Chapter II.). The development of acids may be detected after the addition of 2 per cent. of rosolic acid solution (0.5 per cent. solution in alcohol); alkaline solutions give a clear rose-color which disappears in the presence of acids.

Blood-serum.—The blood of the ox or cow may be obtained easily at the abattoir. It should be collected in a clean jar. When it has coagulated, the clot should be separated from the sides of the jar with a glass rod. It may be left on the ice for from twenty-four to forty-eight hours. At the end of that time the serum will have separated from the clot and may be drawn off with a siphon into tubes. These tubes are sterilized for the first time in a slanting position, as the first sterilization coagulates the serum. The coagulation may be done advantageously, as advised by Councilman and Mallory, in the

hot-air sterilizer at a temperature below the boiling-point. After coagulation, sterilize as usual. This serum makes an opaque medium of a cream color. Blood-serum may be sterilized in the special form of sterilizer devised for it. A clear blood-serum is to be obtained by sterilization at a temperature of 58° C. for one hour, on each of six days, if a fluid medium is desired, or of 75° C. on each of four days if the serum is to be solidified. In the latter case the tubes are to be placed in an inclined position. (See page 55.) Opaque, coagulated blood-serum has most of the advantages of the clear medium. Blood-serum may be secured from small animals by collecting blood directly from the vessels, using very great care to obtain the blood in a sterile condition; and the serum may be separated and stored in a fluid state. Human blood-serum is sometimes obtained from the placental blood, sometimes from serous pleural transudates or from hydrocele fluid. The preservation of blood-serum is sometimes accomplished with chloroform, of which 1 per cent. is to be added to the medium; in this manner the serum may be preserved for a long time. It may be divided into tubes, solidified and sterilized as required; the chloroform is driven off by the heat, owing to its volatility. Blood-serum media which are sterilized at low temperatures should be tested for twenty-four hours in the incubator to prove that sterilization has been effective; if it has not, development of the contaminating bacteria will take place and be visible to the eye.

It will be impossible to do more than merely mention some of the most important of the other culture-media.

Löffler's blood-serum consists of one part of bouillon containing 1 per cent. of glucose, and three parts of blood-serum. It is sterilized like ordinary blood-serum. It is used largely for the cultivation of the bacillus of diphtheria.

Blood-serum-agar is a medium made with considerable difficulty, but very valuable for the cultivation of the gon-

ococcus. One part of placental blood-serum, or pleuritic serum, or hydrocele fluid, is mixed with one to two parts of nutrient agar in the fluid condition. It must be divided into tubes before solidification. Solidify in a slanting position; subsequently sterilize at 75° C. so as not to coagulate the albumen of the blood-serum. The nutrient agar in this case should contain 2 per cent. of dry agar.

Another expedient has also been to smear a little blood, drawn from a puncture made by a sterile needle in the carefully cleaned finger, over the surface of a tube of nutrient agar—*blood-agar*—used for cultivating the bacillus of influenza. Small quantities of blood may be drawn from a vein in the ear of a rabbit (see page 94) with a sterile hypodermic syringe, and is quickly divided among three or four tubes of agar, melted in the upper third; slant the tubes while cooling. To make a large amount of blood-agar, bleed a rabbit from the carotid artery into a sterile flask containing pieces of sterile glass tubing; shake the flask constantly; divide the defibrinated blood among tubes containing sterile nutrient agar; slant the tubes while cooling. Use about one part of blood to about three of agar. Great care must be used not to contaminate the blood as it is drawn. The tubes when completed should stand some days before using, so that contaminating bacteria if present may grow in the interval and permit such tubes to be discarded.

Guarnieri's medium consists of a mixture of gelatin and agar.

Eggs in their shells may be used after sterilization by steam, which of course coagulates the albumen. The egg is easily inoculated through a small opening made with a heated needle, which may be closed afterward with collodion. Egg-albumen has been used as a constituent of various media. Dorset* has found that eggs furnish an excellent culture-medium for tubercle bacilli. The yolk and the white are mixed, poured into tubes, slanted, coagulated, and sterilized. Just before using pour into the tube a few drops of sterile distilled water to moisten the medium. This is a most valuable addition to the technique.

Bread-paste (finely-divided dry bread, mixed with water and sterilized) is used for the cultivation of moulds. *Sabouraud* recommends the following for the cultivation of the trichophyton fungus:

Peptone	5.0 grams.
Maltose	3.8 grams.
Agar	1.3 grams.
Water	100.0 c.c.

Test-tubes.—Bacteria are generally cultivated in test-tubes. A convenient size is one $\frac{5}{8}$ of an inch in diameter

* *American Medicine.* April 5, 1902.

and 5 inches in length. The tubes should be of a heavier glass than in those used for ordinary chemical work. The New York Board of Health, and some others, use a tube three inches in length without a flange for the cultivation of the diphtheria bacillus on Löffler's blood-serum mixture. Test-tubes should be thoroughly cleaned with a swab before using; they should be boiled with washing-soda, rinsed, filled with hydrochloric acid solution, rinsed and inverted to drain away the fluid.

Plugs of raw cotton or cotton batting are employed as stoppers. Some prefer absorbent cotton, but it is likely to become soggy after exposure to steam. The plug should fit smoothly; creases and cracks around the edges are to be avoided. The plug should be tight enough to sustain the weight of the tube when held by the plug. These plugs prevent bacteria from entering or leaving the tubes.

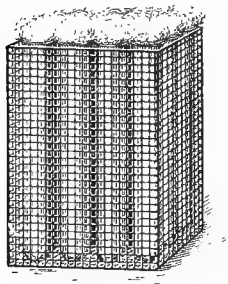

FIG. 18.—WIRE BASKET FOR TEST-TUBES.

Sterilization of Test-tubes.—The tubes are to be sterilized in a hot-air sterilizer for one hour, at a temperature of 150° C. They may be left in until the cotton acquires a light-brown color, but it should not be burned. If the plugs touch the sides of the sterilizer or lie against the bottom they may be scorched.

The necessity for sterilization of the tubes before filling them with the medium has been questioned, and it is probably unnecessary as far as the preservation of the culture-medium is concerned, but it will be found that the cotton plugs fit much better after sterilization with dry heat. During this and subsequent sterilizations the tubes are held in a wire basket.

Filling of the Tubes.—A special funnel closed with a stopcock for filling tubes with liquefied media is often recommended. They may readily be filled with an ordinary funnel of small size.

During the filling, the neck of the test-tube where it comes in contact with the cotton *must not be wet* with the medium. Ordinarily about 7 to 10 c.c. are placed in a test-tube. For Esmarch's roll-tubes a somewhat smaller quantity is desirable.

The sterilization of tubes containing culture-media is always done by steam, and has been sufficiently described. It is to be remembered that the solidifying power of gelatin is impaired by too prolonged heating, while heating is less likely to damage other culture-media. The media which are sterilized at a low temperature (70° C.) should be tested for two days in the incubator to determine whether sterilization has been effective. It is the universal experience in bacteriological laboratories that occasionally culture-media will become contaminated with extremely resistant spores which fail to be sterilized by the ordinary processes, an occurrence which causes great annoyance and calls for the exercise of much patience. Sometimes, also, moulds attach themselves to the plugs, especially if they are moist, and send their filaments down through the cotton; finally, having reached the lower edge of the cotton, their spores may fall upon the medium, grow there and ruin it.

CHAPTER IV.

THE CULTIVATION OF BACTERIA.

Inoculation of the Tubes.—The air of the laboratory should be as quiet as possible, to lessen the chances of contamination by bacteria clinging to particles of dust. Avoid working where there may be draughts or gusts of air or near an open window. Spores are blown from the surfaces of moulds, like thistle-down, and are constantly being wafted about in the air. Given any material containing bacteria, for example a pure culture of some well-known species, a very minute portion is to be introduced into a tube containing the sterile culture-medium. The introduction is effected with a straight platinum wire, or with a platinum wire loop. The platinum is to be heated red-hot before using, and then allowed to cool. It is also to be heated red-hot after using. The plug of the test-tube is to be withdrawn, twisting it slightly, taking it between the third and fourth fingers of the left hand, with the part that projects into the tube pointing toward the back of the hand. It must not be allowed to touch any object while the inoculation is going on. Pass the neck of the tube through the flame. If any of the cotton adheres to the neck of the tube, pull the cotton away with sterilized forceps, while the neck of the tube touches the flame, so that the threads of cotton may be burned and not fly into the air of the room. The tube is held as nearly horizontal as possible. The tube is to be held in the left hand between the thumb and forefinger, the tube resting upon the palm, and the neck of the tube pointing upward and to the right. When two tubes are being used at the same time, as is often necessary, they are placed side by side between the thumb and forefinger of the left hand. The two plugs are held

between the second and third and the third and fourth fingers of the left hand, respectively. The wire may now be passed into the first tube, which we will suppose to hold some material containing bacteria, and a little of this material may be removed on the tip of the wire from the first tube to the second. When the needle is introduced into or removed from either tube it should not touch the side of the tube at any point, and should only come in contact with the region desired. After inoculation of the second tube has been effected the wire is to be heated to a red heat in the flame, the necks of the tubes are to be passed through the flame, and the plugs are to be returned to their

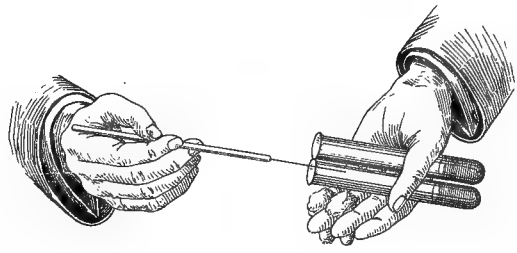

FIG. 19.—MANNER OF HOLDING TUBES.

respective tubes. When the wet wire is to be sterilized in the flame it should be approached to the flame gradually, so as to dry the material on it before burning it, in order to avoid "sputtering" (see page 22). It is well from the start to train one's self to *sterilize the platinum wire every time it is taken from the table and before it is laid down again.* The platinum wire loop may be used in the same manner as the straight wire, especially when a substance containing a small number of bacteria is being handled.

When a tube of gelatin is to be inoculated the wire is usually introduced into the medium vertically, "stab-culture"; when a medium with a slanted surface is employed, as agar, potato

or blood-serum, the needle should lightly streak the surface, "smear culture" (Figs. 20 and 21).

The safety and success of this method of inoculation depend upon a principle which has been established by long and repeated observation,—namely, that bacteria do not of themselves leave a moist surface. They should not, there-

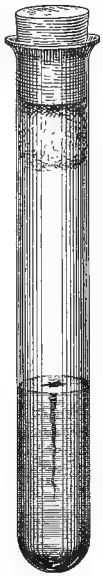

FIG. 20.—STAB CULTURE.
A rubber stopper may be used to prevent drying, see page 79.

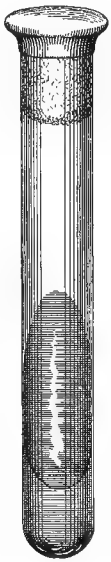

FIG. 21.—SMEAR CULTURE.
This tube shows the rubber cap used to prevent drying.

fore, rise from the surface of the moist culture-medium, nor drop from the needle during its transit, if proper care be exercised. They may be thrown into the air if the needle be allowed to sputter in the flame.

It must be remembered also that such organisms as moulds develop spores which are formed on filaments elevated above the surface of the medium and are easily detached.

If, by any accident, drops of infectious material should fall upon a surface like the table, they should be covered at once with bichloride of mercury solution 1–1000. A good way is to cover the spot with a piece of blotting-paper wet with the solution, place a bell-jar over it and leave for several hours. If infectious material should reach the hands or clothing, they should be thoroughly soaked in the bichloride solution. When working with pathogenic bacteria it is well to wash the hands in this solution and with soap and water, as a routine procedure, before leaving the laboratory.

To maintain their vitality bacteria need to be transplanted from one tube to another occasionally; the time varies greatly with different species. Many bacteria grow on culture-media with difficulty at the first inoculation, but having become accustomed to their artificial surroundings, as it were, they may be propagated easily afterward; this is especially true of the bacillus tuberculosis.

Some bacteria flourish better on one culture-medium than on another. The bacillus tuberculosis grows best on blood-serum and glycerin-agar; the bacillus of diphtheria grows best on Löffler's blood-serum; the gonococcus on human serum-agar.

The virulence of most pathogenic bacteria becomes diminished after prolonged cultivation upon media. Sometimes the virulence is lost very quickly,—for example, the streptococcus pyogenes and micrococcus lanceolatus of pneumonia.

Incubators.—Many bacteria flourish best at a temperature about that of the human body, 38° C. Some species will grow only at this temperature. The pathogenic bacteria in particular, for the most part, thrive best at a point near the body temperature.

The incubator is a box made of copper, having double walls, the space between the two being filled with water. The outer surface is covered with some non-conductor of

heat, such as felt or asbestos. At one side is a door, which is also double. The inner door is of glass, the outer door is of copper covered with asbestos. At one side is a gauge which indicates the level at which the water stands in the

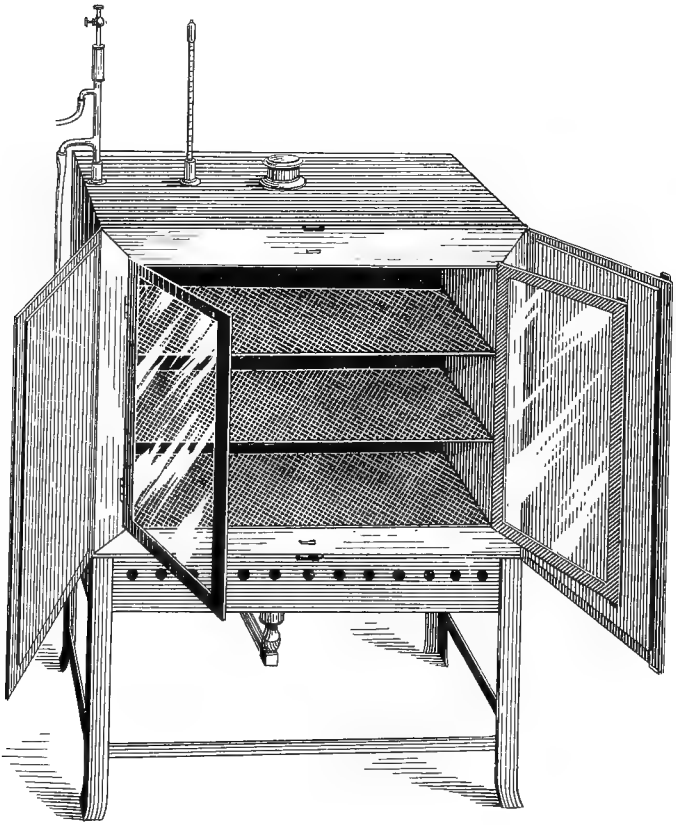

FIG. 22.—INCUBATOR.

water-jacket. The roof is perforated with several holes, some of which permit the circulation of the air in the air-chamber inside the box; some of them enter the water-jacket. A thermometer passes through one of these holes into the

interior of the air-chamber, and often another into the water standing in the water-jacket. A gas-regulator passes through another hole, and is immersed in the water standing in the water-jacket. There are various forms of gas-regulators more or less complicated. In general they consist usually of a tube containing mercury; into this tube are two openings, one

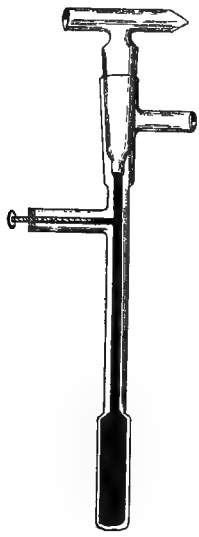

FIG. 23.—REICHERT'S GAS-REGULATOR.

FIG. 24.—MERCURIAL GAS-REGULATOR.

a. Chamber containing volatile hydrocarbon. *b*. Capillary opening.

for the entrance and the other for the exit of gas. The gas enters through a small tube, which is cut off diagonally at the bottom, and which projects into the surface of the mercury. Heating the water in the water-jacket causes expansion of the mercury, which rises, and, little by little, cuts off the inflow of gas through this tube. The flow is never completely cut off, as there is a capillary opening in the tube consider-

ably above any point to which the mercury could possibly rise, which will always allow the flow of a small quantity of gas (Fig. 24, *b*). This diagram also shows a modification of the simple form of regulator, in the shape of a partition which divides off a lower chamber, which contains mercury and is connected with the upper part by a glass tube. The purpose is to make use of the elastic properties of some volatile fluid, like ether, which floats on the surface of the mercury at *a*. The gas coming from the gas-regulator passes to a Bunsen burner, which stands underneath the incubator. This burner should have some kind of automatic device for cutting off the flow of gas in case it becomes accidentally extinguished by a sudden draught of air or from any other cause. The automatic burner invented by Koch is an ingenious, simple and effective device. A bar of metal stands above the flame; by its expansion, through a system of levers, it supports a weight; the weight controls a gas-cock. While the flame is burning the expansion of the metal holds the weight horizontally; if the flame becomes extinguished, the metal contracts, the weight falls and cuts off the flow of gas. Some inconvenience will arise from irregularities in the flow of gas from the main supply-pipe. Any incubator will vary a little from such causes. In the experience of the writer, natural gas is of such variable pressure as to be entirely useless. Fluctuations of the temperature within the incubator depend very largely upon the external temperature. Therefore the incubator should, as far as is

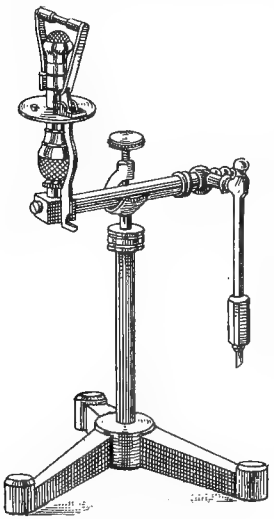

FIG. 25.—KOCH AUTOMATIC GAS-BURNER.

practicable, be protected from sudden draughts of cold air and should be kept in a room having as equable a temperature as possible. In large laboratories it is often found convenient to use the whole of a small room as an incubator, heating it by a gas stove, to which a gas regulator may be applied.

Culture-tubes which are being kept in the incubator are likely to become dry if their stay is prolonged. In such cases they should be covered with rubber caps, tin-foil, sealing-wax, paraffin, or some other device to prevent evaporation. If rubber caps are used, they should be left in 1–1000 bichloride of mercury solution for an hour, and the cotton plugs should be singed in the flame, before putting them on. (Fig. 21.) The writer prefers rubber stoppers, which may be boiled and stored in bichloride of mercury solution. Cut the cotton plug even with the edge of the tube, singe it in the flame, push it into the tube about 1 cm. and insert the rubber stopper. (Fig. 20.)

Cultivation of Anaërobic Bacteria.

The cultivation of anaërobic bacteria is done best in a medium containing 1 to 2 per cent. of dextrose. The tube should contain a large quantity of the culture-medium. Just before using, the medium should be boiled for a few minutes. Inoculate the tube after cooling, but while the medium is fluid. Anaërobes may be cultivated in the closed arm of the fermentation-tube (see Fig. 46), but the opening between the two arms of the tube must be small.

Buchner's Method for the Cultivation of Anaërobes: Into a bottle or tube which can be tightly stoppered, pour 10 c.c. of a 6 per cent. solution of sodium or potassium hydroxide, for each 100 c.c. of air contained in the jar. Add one gram of pyrogallic acid for each 10 c.c. of solution. The culture-tube is placed inside of the larger bottle or tube, supported

above the bottom, and the stopper, smeared with paraffin, is inserted. The mixture of pyrogallic acid and potassium hydroxide possesses the property of absorbing oxygen.

Wright's Modification of Buchner's Method: The tube of culture-medium is to be plugged with *absorbent cotton*, using a plug of large size. The culture-medium is inoculated in the usual way. The plug is cut off close to the neck of the tube, and is then pushed into the tube about 1 centimeter. Now allow a watery solution of pyrogallic acid to run into the plug, and then a watery solution of sodium or potassium hydroxide. Close quickly and tightly with a rubber stopper. Wright recommends that the first solution be freshly made and consist of about equal volumes of pyrogallic acid and water, and that the second solution contain 1 part of sodium hydroxide and 2 parts of water. With 6 inch test-tubes, $\frac{3}{4}$ inch diameter, the amounts advised are—$\frac{1}{2}$ c.c. solution of pyrogallic acid, 1 c.c. solution of sodium hydroxide.

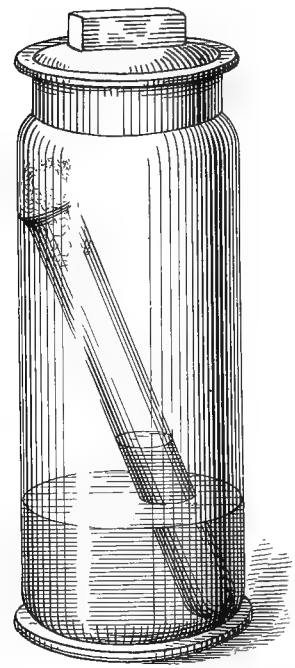

FIG. 26.—ARRANGEMENT OF TUBES FOR CULTIVATION OF ANAËROBES BY BUCHNER'S METHOD.

Cultivation of Anaërobic Bacteria under Hydrogen: Method of Liborius Modified by Fränkel: A test-tube containing a large amount of the liquefied culture-medium is closed with a sterilized rubber stopper, through which pass two sterilized glass tubes, bent above the stopper at a right angle. One of these

tubes is cut off just underneath the stopper, and the other is long enough to project nearly to the bottom of the culture-tube. The horizontal projecting parts are drawn to a small caliber at some point, although not quite closed, to facilitate sealing later on. Through the longer of these tubes hydrogen gas is passed until the atmosphere inside of the culture-tube is pure hydrogen, entirely free from mixture with air. The horizontal parts of the small glass tubes projecting from the stopper are then sealed in the flame at the places where they were previously drawn out to a small caliber, and the tubes are thus closed. (Fig. 27.)

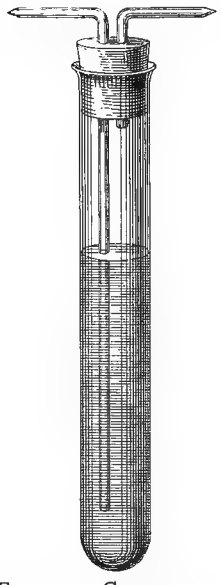

FIG. 27.—CULTIVATION OF ANAËROBES BY FRÄNKEL'S METHOD.

The stopper should be surrounded with melted paraffin. A tube prepared according to this plan may, if desired, be converted into an Esmarch roll-tube. The hydrogen is generated according to the common method with *pure* zinc and *pure* sulphuric acid, 25 to 30 per cent. The precautions advised by chemists for the generation of hydrogen must be carefully followed, because when hydrogen mixed with oxygen or air is ignited a violent and disastrous explosion may occur.

The well-known Kipp's generator may be used. First let the reservoir fill with hydrogen; then allow its contents to escape. This should be repeated, after which some of the hydrogen may be collected in an inverted test-tube under water. When this sample is ignited, it should burn without any explosion; otherwise the hydrogen is not yet ready to use. The hydrogen should bubble through the medium five minutes or more.

The inconvenience and danger of sealing the tubes in the flame, as has to be done in Liborius-Fränkel's and other methods for cultivation under hydrogen, are obviated in *Novy's apparatus*. The tubes or plates are placed in jars through which hydrogen may be conducted. The stopper, having been smeared previously with a soft wax, is sealed by giving it one-fourth of a turn.

There have been various other kinds of apparatus, usually complicated and expensive, devised for the growth of plate-cultures under hydrogen, but Novy's jars are the best, both for tubes and for plates.

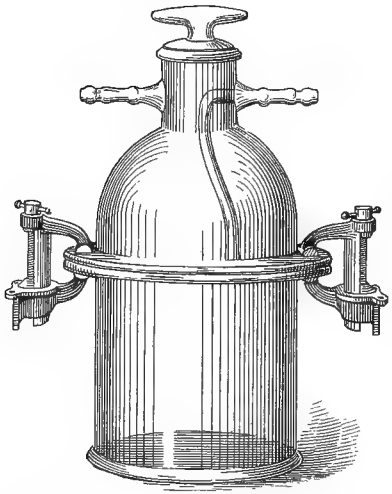

FIG. 28.—NOVY'S JAR FOR THE CULTIVATION OF ANAËROBES.

Other expedients for the cultivation of anaërobic bacteria are less effective. In cases where a very deep stab-culture is made in gelatin or agar, where the growth appears in the lower part of the tube by preference, it is supposed to be anaërobic. Koch covered part of the surface of a gelatin plate with a bit of sterilized mica or a cover-glass; bacteria which grew beneath this plate were considered to be anaërobic. Another method was to cover the surface of the gelatin in the culture-tube with sterilized oil. W. H. Park has recommended a mixture of solid paraffin with 25 to 50 per cent. of fluid paraffin or albolene as a covering for the surface of anaërobic cultures. This mixture has a semisolid consistency, and does nor retract at the edges on cooling. The paraffin prevents the absorption of oxygen, except to a small extent at the edges. The method is useful for large quantities of culture material, as in flasks. Esmarch advised making roll-tubes, and after cooling them to fill them with a melted gelatin cooled down to near the point of solidification. Hueppe made use of eggs in their shells. The egg-shell was carefully cleaned, sterilized with a solution of bichloride of mercury, washed with sterilized water and wiped dry with sterilized cotton. The end of the egg-shell was punctured with a hot needle

Through the opening thus made the inoculation was accomplished. The opening was closed with collodion.

FIG. 29.—STREAK CULTURE OF THE POTATO BACILLUS (NATURAL SIZE), SHOWING AN AËROBIC ORGANISM WHICH WILL NOT GROW UNDER A COVER-GLASS.

CHAPTER V.

CULTIVATION OF BACTERIA, CONTINUED.

Isolation of Bacteria.—In order to study any kind of bacteria it is necessary to have the particular species separated from other sorts with which it may be mixed. The earlier bacteriologists endeavored to separate bacteria of different sorts by successive transplantations through a series of tubes. The procedure now generally used for this purpose is the so-called plate-method of Koch. The great progress which bacteriology has made during the last twenty years is largely owing to the use of this method.

Pathogenic bacteria may sometimes be isolated through inoculations into animals. Thus an animal may be inoculated with sputum containing tubercle bacilli mixed with other bacteria. The animal may die of tuberculosis, and its tissues may contain tubercle bacilli in pure culture, the other bacteria having produced no important effect.

Still another method which is occasionally useful is to subject the mixture of bacteria to a heat of 80° or 90° C. for a few minutes. If it contains resistant spores, like those of the tetanus bacillus or hay bacillus, they may be expected to survive, and may be propagated in pure culture, everything else having been killed by the heat.

Plate-cultures.—It is impossible in most cases to distinguish between bacteria of different varieties by microscopic examination alone. Bacteria of widely different species and quite unlike one another in their properties may present similar appearances under the microscope. The differences which

they exhibit are usually apparent when they are grown in culture-media. The growth, called a *colony*, which results from the multiplication of a single bacterium, is in many cases quite characteristic for the species. By the plate-method the individual bacteria in a mixture are separated from one another by distributing them through melted gelatin or agar in tubes. They are fixed in the place where they chance to be when the medium solidifies. They are allowed to grow, and from each individual there forms a colony. It is usually possible to distinguish between *colonies* arising from different species when it was not possible to distinguish between *the individual bacteria* of these species. A convenient illustration has been suggested by Abbott. A number of seeds of different sorts may appear very much alike, and considerable difficulty may be found in distinguishing one from another with the eye. Let them be sown, however, and let plants develop from them, and these plants will easily be distinguished from one another.*

Method of Making Plate-cultures.—Melt *three* tubes of gelatin or agar. (There is some difficulty in keeping agar in a fluid state while dilutions are being made. It is best to have some form of water-bath with a thermometer for the purpose.) Let the liquefied tubes cool to a few degrees above 40° C. Take a small portion of the material to be examined—pus, for example—and introduce it with a sterilized platinum wire or loop into one of the tubes. Stir it in carefully. Remove the needle, sterilize it and replace the plug. Mix the material introduced thoroughly with the melted culture-medium, taking care not to wet the plug. Now remove the plug again, and, having sterilized the platinum wire, insert it into the liquefied medium. Carry three loopfuls in succession from this tube, which is No. 1, into tube No. 2; sterilize the needle; replace the plugs; mix

* It must be understood that *no close comparison can be drawn between higher plants*, which simply complete the development of parts potentially present in the seed, *and colonies of bacteria*, which are aggregates of individuals the progeny of one individual of the same kind.

thoroughly, without wetting the plug. Carry three loopfuls from tube No. 2 into tube No. 3 in the same manner. The original material will obviously be diluted in tube No. 1, more in tube No. 2 and still more in tube No. 3. The most convenient form of plate is that known as a Petri dish, a small glass dish about 8 cm. in diameter and 1.5 cm. in height, provided with a cover which is a little larger, but of the same form. This dish should be cleaned and sterilized in a hot-air sterilizer at 150° C. or higher for an hour. When it is cool it may be used.

Such dishes having previously been prepared, the contents of tube No. 1 are poured into one dish, and those of tube No. 2 into another and those of tube No. 3 into a third. They are to be labeled Nos. 1, 2 and 3.* In pouring proceed as follows:

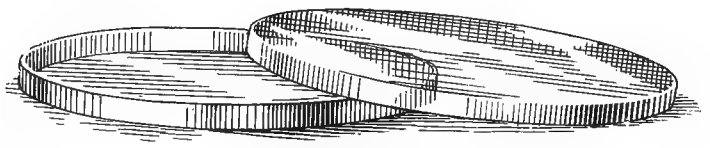

FIG. 30.—PETRI DISH.

Remove the plug of tube No. 1; heat the neck of the tube in the flame; allow it to cool, holding it in a nearly horizontal position. When the tube has cooled, lift the cover of the Petri dish a little, holding it over the dish; pour the contents of tube No. 1 into the dish and replace the cover of the dish. The interior of the dish should be exposed as little and as short a time as possible. Tubes Nos. 2 and 3 are to be treated in the same manner. Burn the plugs, and fill the empty tubes with 5 per cent. solution of carbolic acid. They should be sterilized for an hour in the steam sterilizer on each of three days.

The culture-medium in the Petri dish will soon solidify.

* The labels should be moistened with the finger, which has been dipped in water. They should not be licked with the tongue. While working in the bacteriological laboratory it is best to make it a rule that no object is to be put in the mouth.

Petri dishes of agar should be inverted as soon as the medium is thoroughly solidified, otherwise the water which evaporates

FIG. 31.—DILUTION-CULTURES IN ESMARCH ROLL-TUBES.
In tube 1 the colonies are very close together; in tube 2 they are somewhat separate; in tube 3 they are well isolated.

from the surface condenses on the inside of the lid, and runs

down over the surface of the agar. A round piece of filter paper placed over the dish before putting on the lid may also be employed, or the cover may be made of porous earthenware, as recently recommended by Hill. Colonies develop usually in from one to two days, more quickly, of course, in the incubator. In plate No. 1 they will be very numerous, in plate No. 2 less numerous and in plate No. 3 still less numerous. Where the number is small the colonies will be widely separated and can readily be studied. They may be examined with a hand-lens,

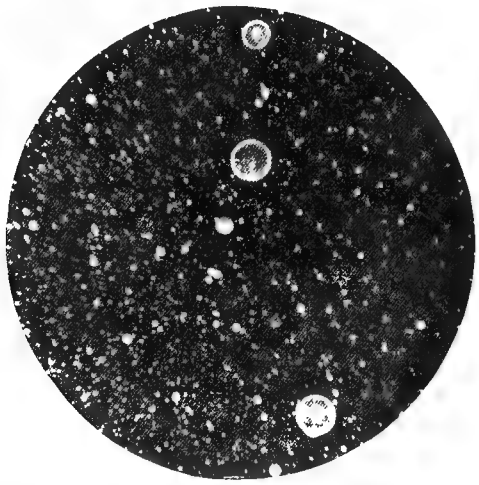

FIG. 32.—APPEARANCE OF COLONIES ON GELATIN IN PETRI DISH.

or the entire dish may be placed on the stage of the microscope and the colonies be inspected with the low power. The iris diaphragm should be partly closed and the concave mirror should be used. Dilution-cultures prepared as described in the next paragraph, where the principle is the same, are shown in Fig. 31. In tube No. 1 the colonies are so numerous as to look like fine, white dust. In tubes 2 and 3 they become less numerous and larger.

Esmarch's Roll-tubes.—Either gelatin or agar may be used, but if the agar is freshly made, it does not adhere well to the walls of the tubes. The dilutions in tubes 1, 2 and 3 are made as above. Tubes containing a rather small amount of the culture-medium are more convenient. A block of ice should be at hand, and, with a tube filled with hot water and lying horizontally, a hollow of the size of the test-tube should be melted on the upper surface of the ice. In this hollow place the tube of liquefied gelatin or agar; roll it rapidly with the hand, taking

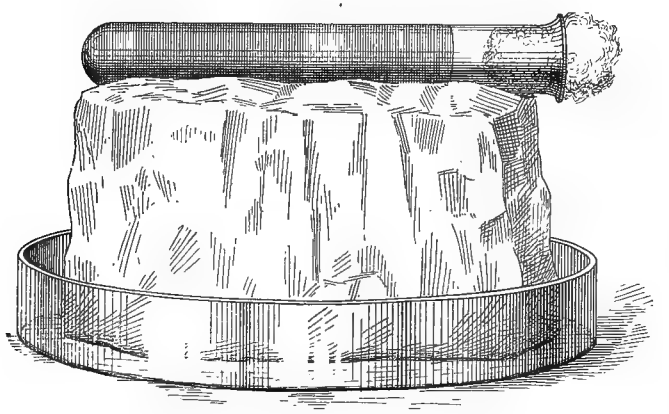

FIG. 33.—MANNER OF MAKING ESMARCH ROLL-TUBE.

care that the culture-medium does not run toward the neck as far as the cotton plug. The medium is spread in a uniform manner around the inside of the tube, where it becomes solidified. Gelatin roll-tubes must be kept in a place so cool that there is no danger of their melting; in handling them they are to be held near the neck, so that the warmth of the hand may not melt the gelatin. Agar roll-tubes should be kept in a position a little inclined from the horizontal, with the neck up, for twenty-four hours, so that the agar may stick to the wall of

the tube. For reasons stated above, it is best to employ agar which has dried out partially.

By the plate-method as originally devised by Koch, instead of using Petri dishes, the gelatin was poured upon a sterile plate of glass. This plate of glass was laid on another larger plate of glass, which formed a cover for a dish of ice-water, the whole being provided with a leveling apparatus. The plate was kept perfectly level until it had solidified, which took place rapidly on the cold surface. The glass plates were placed on little benches enclosed within a sterile chamber. The more convenient Petri dish has displaced the original glass plate to a large extent.

The isolation of bacteria may sometimes be effected by drawing a platinum wire containing material to be examined rapidly over the surface of a Petri dish containing solid gelatin or agar; or over the surface of the slanted culture-medium in a test-tube; or by drawing it over the surface of the medium in one test-tube, then, without sterilizing, over the surface of another, perhaps over several in succession.

Another very convenient method of obtaining isolated colonies is to introduce a very small amount of material into the water squeezed out in the bottom of a slant agar tube, then flood this over the surface of the agar.

Appearance of the Colonies.—The colonies obtained in the Petri dishes or roll-tubes (Fig. 32) may be studied with a hand-lens or with a low power microscope. In the latter case, use the concave mirror with the iris diaphragm partly closed. The colonies present various appearances. Some of them are white, some colored; some are quite transparent and others are opaque; some are round, some are irregular in outline; some have a smooth surface, others appear granular, and others present a radial striation. Surface colonies often present different appearances from those occurring more deeply. Surface colonies are likely to be broad, flat and spreading. If the colony consists of bacteria which have the property of liquefying gelatin, a little funnel-shaped pit or depression forms at the site of the colony. The appearance of colonies may be of great as-

sistance in determining the character of doubtful species. The appearance in gelatin plates of the colonies of the spirillum of Asiatic cholera, for instance, is one of the most characteristic manifestations of this organism.

Pure Cultures.—From these colonies pure cultures may be obtained by what is called "fishing." Select a colony from which cultures are to be made; touch it lightly with the tip of a sterilized platinum wire, taking great care not to touch the medium at any other point. Introduce the wire into a tube of gelatin. Sterilize the wire and plug the tube. In a similar manner, and from the same colony, inoculate tubes of agar, bouillon, milk, potato and blood-serum. At the same time it is well to make a smear preparation from the colony and to stain with one of the aniline dyes so as to determine the morphology of the bacteria. The growths which take place in the tubes should contain one and the same kind of bacteria. As seen under the microscope their bacteria should have the same general form and appearance as those seen in the colony from which they were derived. This will be the case, provided the colony has resulted from the development of a single bacterium or from several bacteria of the same kind. Occasionally, however, a colony will develop from several bacteria which may not all be alike. In that case a pure culture will not be obtained, and the process of plating may have to be repeated.

CHAPTER VI.

INOCULATION OF ANIMALS.

In the study of pathogenic bacteria, the inoculation of animals is frequently indispensable. It is inexpedient where classes are large for students to make such inoculations; but, nevertheless, every student should be familiar with the subject. The animals most often used are white mice, guinea-pigs, rabbits, and pigeons. Larger animals are occasionally employed for special purposes. White mice may be kept in a glass jar covered with wire netting. They may be fed with moistened bread or oats. It is important to see that they receive drinking-water. During

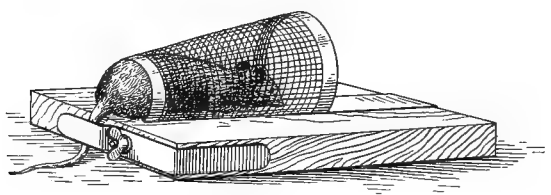

FIG. 34.—MOUSE-HOLDER.

inoculation the mouse must be kept in position by some sort of mouse-holder, or may be held by an assistant, who takes the skin at the back of the neck between his fingers and at the same time holds the tail. The hair is cut off from the skin at the root of the tail. A small V-shaped opening in the skin is made with scissors, and a stiff, sterilized, platinum wire is passed into this opening, separating the skin from the muscles for some distance so as to make a pocket. Into this pocket the material is introduced by means of the platinum wire. The wound may be covered with collodion.

Guinea-pigs and rabbits, after inoculation, are to be kept in cages of galvanized iron and wire-netting. The bottom may conveniently be made in the form of a movable pan which permits of the disinfection of the excreta. Rabbits and guinea-pigs may be fed with oats, carrots, cabbage, grass and the like. Guinea-pigs and rabbits may be held by an assistant or tied by the legs upon a board. The hair over a small portion of the abdomen is cut away and a short incision is made through the skin; a pocket is produced with a stiff wire, and the material inserted with a sterile platinum wire. The wound may be covered with collodion. Sutures may be used if the wound is large. Solid substances may conveniently be introduced by placing them in a sterile glass cannula, which is pushed to the proper situation through a small incision. The substance in the cannula is forced out of it with a stiff, sterile, platinum wire. (Fig. 35.)

FIG. 35.

The peritoneal cavity may be inoculated through an incision in the abdominal wall, into which the desired substance may be introduced with a sterile platinum wire, the incision being closed with sutures.

But a more convenient method in many cases, both for subcutaneous as well as intraperitoneal inoculations, is the use of a hypodermic syringe. Material from the surface of solid media can be suspended in sterile beef-broth or physiological salt solution, and cultures in fluid media used directly for these injections.

Intravenous inoculation is most commonly practiced upon

rabbits. A small vein which is near the posterior margin of the ear of the rabbit is easily reached from the dorsal surface; the ear having been shaved and washed with alcohol, the hypodermic needle is introduced directly into this vein. In making a hypodermic injection, the needle and syringe should of course be sterilized before and after each operation.

Autopsies upon animals should be held as soon as possible after death. During the interval the body should be kept in the ice-box. The autopsy room should be furnished with screens to keep out flies, so that they may not light on the infected animal. The animal should be extended on its back upon a board. The legs may be fastened with pins or tacks. The animal should be handled with forceps as far as possible, and after beginning the autopsy the fingers should not touch it. If the fingers come in contact with infectious matter, disinfect them at once. Have a basin of bichloride of mercury solution 1–1000 ready for this purpose. Knives, scissors, platinum wires and forceps should be sterilized in the flame before and after each manipulation. Be prepared to make smear preparations on cover-glasses, and to inoculate tubes of gelatin, agar and other media as desired. Moisten the hairs over the thorax and abdomen with bichloride of mercury solution 1–1000, to prevent them from being carried into the air. Make an incision, passing through the skin from the sternum to the pubis along the thorax and abdomen, and diagonal incisions extending down the fore and hind legs. Dissect away the skin from the thorax, abdomen and upper parts of the legs. With a knife heated in the flame, sear a broad line extending down the middle of the abdomen. Through this burned surface make an incision through the muscles of the abdomen. In a similar manner make a transverse incision across the middle of the abdomen through a burned surface. Cultures should be made from the peritoneal cavity, and smears upon cover-glasses prepared, which are afterward to be stained. With a hot knife, scorch a small area on

the surface of the liver; through this surface enter the liver with a sterilized, platinum wire, and with the material thus obtained inoculate the tubes; also make cover-glass preparations. In the same manner inoculate tubes and make cover-glass preparations from the spleen, the kidneys, the pleural cavity, the pericardial cavity, the lungs, and the blood inside the heart and other organs as indicated. If there is a question of the tissues from which the cultures are to be made having become contaminated, as might be the case where the autopsy is delayed for any reason, it is better to make plates. All incisions are to be made through the burned surfaces, and all material collected for inoculation is to be obtained through burned surfaces. In sterilizing the instruments in the flame avoid sputtering, es-

FIG. 36.—METHOD OF MAKING COLLODION CAPSULES.—(*After McCrae.*)

pecially when they become covered with oil from adipose tissue. Pieces of lung, liver, spleen, kidney and other organs, as may be indicated, should be placed in 95 per cent. alcohol or 10 per cent. formalin for fixation and hardening. The animal and the board on which it was extended should be covered with bichloride of mercury solution 1–1000, and afterward burned. The cage or jar and the instruments, dishes and towels used should be sterilized by steam. The hands of the operator should be washed thoroughly with soap and water and with a 1–1000 solution of bichloride of mercury, if there is any possibility of these having accidentally come in contact with any of the diseased tissues.

Collodion Capsules.—Bacteria may be cultivated in the living body of an animal, without infecting the animal, when they are enclosed in collodion capsules. Their soluble products

are able to diffuse through the collodion, while the animal's fluid may pass into the sac to nourish them. These capsules were originally made by dipping the round end of a glass rod into collodion repeatedly. McCrae's method* is easier and more satisfactory. (Fig. 36.)

A piece of glass tubing is taken, and a narrow neck drawn on it near one end. This end of the tube is rounded in the flame, and the body of a gelatin capsule is fitted over it, while still warm, so that the gelatin may adhere to the glass. The capsule is now dipped into 3 per cent. collodion, covering the gelatin and part of the glass. It is allowed to dry a few minutes, and is dipped again. In all, two or three coatings may be given. The capsule is filled with water and boiled in a test-tube with water. The melted gelatin is removed with a fine pipette. The capsule is partly filled with water or broth and sterilized. The capsule may now be inoculated. The narrow part of the neck must then be sealed in the flame, taking care that the neck be dry. The sealed capsule should be placed in bouillon for twenty-four hours. No growth should occur outside the capsule if it is tight. It may now be placed in the peritoneum of an animal.

A method for making collodion sacs recommended by Gorsline† consists in the use of a glass tube, the lower end of which is rounded and closed, except a small hole, which is temporarily filled with collodion. This tube is dipped in collodion and dried, as above. It may now be filled with water. By blowing at the opposite end, the pressure through the hole in the bottom of the glass tube will cause the capsule to loosen so that it comes away easily.

There are also various other methods recommended for making collodion sacs.

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* *Journal of Experimental Medicine.* Vol. VI., p. 635.

† See Contributions to Medical Research. Dedicated to Victor C. Vaughan. 1903.

CHAPTER VII.

COLLECTION OF MATERIAL.

Samples of water or milk should be examined as soon after drawing as possible; but when this is impossible, as in the case where they are transmitted from a distance, they should be collected in sterilized tubes or bottles, which should be kept on ice. Specimens of sputum should be collected in clean bottles tightly corked. The early morning sputum is to be preferred for examination. The patient should be directed to rinse out the mouth carefully, and cough up material from the lungs, not merely to clear out the throat as is sometimes done. It should be examined as soon as possible. Although decomposition appears not to interfere with the staining properties of the tubercle bacilli, the sputum should be fresh in order that the other bacteria contained in it may be studied. Therefore it should be free from contamination with putrefactive germs. Valuable information can also be obtained by examination of sputum in a fresh condition before staining (see also page 33).

Samples of urine keep better after the addition of a few crystals of thymol, which retards the fermentative process, so that the sedimentation of the bacteria and of other solid matter in conical vessels is facilitated, although that purpose can be accomplished at once by the centrifuge. Thymol will also be a useful addition, as far as a bacteriological examination is concerned, in case samples of urine are to be sent by mail; thymol should not be added if cultures are to be made.

Specimens of sputum, pus or blood may be collected conveniently in the form of thin smears upon cover-glasses. The smears are fixed by passing through the flame three times.

Smears of blood are prepared as follows: Have two perfectly clean, square cover-glasses. The finger, or the lobe of the ear, having been carefully washed with water, alcohol and ether, is punctured with a sterilized needle, and a small drop of blood issues which is wiped away with a clean cloth. The second drop of blood should be taken; it should be about the size of a pin's head. No pressure should be exerted upon the skin. This drop of blood is placed on one of the cover-glasses. The other cover-glass is laid upon the first, both being handled with forceps. The drop of blood becomes flattened out into a thin film. Immediately and before the blood has had time to coagulate the two are slipped or slid away from each other in a horizontal plane, not forcibly pulled apart. In this way the blood will be spread in thin films on the cover-glasses. It is best to place the cover-glasses so that one does not cover the other exactly, but so that the sides of the one lie diagonally to the sides of the other, although their centers coincide (Fig. 37). Films of blood which are to be examined for the parasite of malaria may be prepared in this manner. Samples of blood to be used for the serum reaction for typhoid fever need to be good-sized drops of blood, which may be collected on cover-glasses or pieces of unsized paper and allowed to dry. To test blood by culture methods, 1 to 5 c.c. may be drawn from a vein during life, using a sterilized hypodermic syringe and all antiseptic precautions. The blood thus taken may then be used for cultures in various ways. A good method for general purposes is to empty the syringe quickly into a flask holding 100 c.c. or more of bouillon or dextrose-bouillon. The mixture of blood and bouillon should be placed in the incubator for one to two days. If the bacteria develop, they may be secured in pure

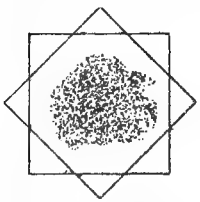

FIG. 37.—MANNER OF PLACING COVER-GLASSES IN MAKING FILMS OF BLOOD.—(*After Cabot.*)

cultures by plating, and may be studied further, as the occasion requires.

At autopsies on human subjects the same principles apply as in the case of autopsies upon animals (see pages 94 and 95). Plate-cultures should be made, if possible, directly from the organs. In all cases organs should be entered by the platinum wire through burned surfaces. The method of isolation by streaking the platinum wire containing the material under examination lightly, several times, over the surface of an agar plate, will be found convenient. At the same time smears should be made from the organs upon cover-glasses for microscopic study, and portions of the organs should be saved and hardened in alcohol or formalin.

A convenient device for the collection of infected material is a stiff wire wound with a pledget of absorbent cotton at one end, the whole sterilized in a tube, as recommended by Warren for collecting pus and other fluids for examination, and as introduced by W. H. Park for the collection of material from the throat in cases of suspected diphtheria (Fig. 78).

FIG. 38.—STERNBERG BULB.

The so-called Sternberg bulb* is valuable for the collection of fluid materials for examination. A piece of glass tubing is taken and drawn out to a long, fine tube, and a bulb blown at the other end. To introduce the substance into the bulb, the expanded end is heated in the flame; the point introduced below the surface of the fluid which is to be collected; as the bulb cools, the air in it contracts and draws the fluid into it. When it has taken up as much as it will, the point may be sealed off in the flame.

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*These bulbs were first recommended by Flügge. Die Mikroöganismen. 1 Auflage, p. 662. 1886.

It should be so packed that breakage or leakage is impossible, particularly when infectious material is to be transported.

Concerning the transmission of materials containing bacteria in the mails, the ruling of the post-office department of the United States, March 2, 1900, is as follows:

"That the order of the Postmaster General of December 27, 1897 (Order No. 677), amending Order No. 88 of February 5, 1896, prescribing the conditions under which specimens of diseased tissues may be admitted to the mails is hereby further modified in the following manner:

"Specimens of diseased tissues may be admitted to the mail for transmission to United States, State, or municipal laboratories, only when enclosed in mailing packages constructed in accordance with the specifications hereinafter enumerated: Liquid cultures, or cultures of microörganisms in media that are fluid at the ordinary temperature (below 45° C. or 113° F.) are unmailable. Such specimens may be sent in media that remain solid at ordinary temperatures.

"Upon the outside of every package shall be written or printed the words 'Specimen for Bacteriological Examination. This package to be treated as letter mail.' No package containing diseased tissue shall be delivered to any representative of any of said laboratories until a permit shall have first been issued by the Postmaster General certifying that said institution has been found to be entitled, in accordance with the requirements of this regulation, to receive such specimens."

The regulation includes not only cultures, but "specimens of diseased tissues." The specifications prescribing the manner of packing, which are minute and complicated, may be obtained from local postmasters.

CHAPTER VIII.

SYSTEMATIC STUDY OF SPECIES OF BACTERIA.

In order to conduct the study of any species of bacteria it is necessary to have the organism isolated in a pure culture. This is best accomplished by the plate method already described. Having thus obtained the organism in pure culture, it is to be examined with reference to its behavior in certain particulars. It is well for the beginner to study a few known species of saprophytes obtained from some reliable laboratory in pure culture. The points which are to be considered can be illustrated best by presenting them in tabular form, filling out the items of the table for a given species of bacteria.

1. Name.
2. Habitat or source.
3. Morphology; grouping, as in chains or in zoöglœæ.
4. Size.
5. Staining properties. Behavior by Gram's method.
6. Capsule, present or otherwise.
7. Spore formation.
8. Motility, flagella.

Growth on culture-media.

9. Relation of growth to temperature.
10. Gelatin; observe whether the gelatin is liquefied or not. Colonies in gelatin plates, study under low power of microscope.
11. Agar. Colonies in agar plates, study under low power of microscope.
12. Bouillon; note cloudiness, pellicle, or precipitate.
13. Milk; observe whether or not the milk is coagulated and subsequently peptonized.

14. Production of gas in fermentation-tube with bouillon containing sugar, as dextrose, or in agar with sugars.
15. Potato.
16. Blood-serum; observe whether or not peptonization occurs.
17. Production of indol.
18. Pigment formation.
19. Production of acid or alkali.
20. Relation to oxygen; observe whether the superficial or the deep part of the growth is the more luxuriant in stab-cultures; use anaërobic methods if necessary.
21. Pathogenesis.

In commencing the study of bacteriology the pupil should try the common staining methods and make the most important culture-media. Having culture-media prepared, it is customary to study a number of species of non-pathogenic bacteria. Notes of the work and sketches showing the morphology of the organisms should be made. In this as in other work with the microscope, the value of even crude drawings is very great as a matter of training. It is well to choose species which have properties decidedly different from one another. The micrococci, bacilli and spirilla should be represented; forms that are motile and that are not; species that form spores and others that do not form spores; some that liquefy gelatin and some that do not. There should be chromogenic forms, and species that ferment dextrose, and that produce indol,—such species as some of the sarcinæ, the bacillus coli communis, the hay bacillus, the potato bacillus, bacillus prodigiosus, a bacillus fluorescens and spirillum rubrum. It is well, when possible, to obtain material directly from nature rather than from laboratory cultures. This may readily be done in the case of the hay bacillus and the potato bacillus. Fecal matter may be spread on gelatin plates and the bacillus coli communis obtained in pure culture. Fluorescing bacilli are very common in water. Large spirilla are often found

in swamp water. Some organisms like spirillum rubrum can only be had from laboratory cultures. An instructive experiment which any one may carry out is to boil a potato thoroughly, and cut it into slices, placing these on moist filter paper on glass plates, or on saucers, and after exposing them to the air for a half an hour or more to cover them each with an inverted tumbler. Some of the slices prepared in this way should be put in the incubator, others left at room temperature. In a shorter or longer time there usually develops a great variety of isolated colonies from the bacteria that have fallen on the slices of potato. The growth of some aërobic organism, like the potato bacillus, may be tested under a cover-glass (see Fig. 29). The pyogenic bacteria, which can easily be isolated from pus, may be studied in this connection with great advantage. The staphylococcus pyogenes aureus and the streptococcus pyogenes should on no account be omitted. The diplococcus of pneumonia can most readily be obtained from a mouse or a rabbit which has died with pneumococcus infection. Such an animal can best be infected by subcutaneous inoculation, using some of the rusty sputum of a case of lobar pneumonia. The cultivation of the pneumococcus will be found to present difficulties in classes containing large numbers of students.

Representative forms of moulds and yeasts should be studied at the same time. Moulds are easily obtained by exposing some nutrient substance to the air, covering it, and allowing cultures to develop; yeasts will probably grow also. Ordinary brewer's yeast may be isolated in pure culture from gelatin plates. Bacteriological examinations also should be made of air, soil, water and milk. With such simple means, many of the important properties of bacteria may be demonstrated. It is most important that medical students should convince themselves by experiment of the extent to which bacteria are disseminated in our environments. The bearings of such observations on the practice of surgery and hygiene are obvious.

Experiments in sterilization and disinfection as described in Chapter VIII., Part II., may be performed with the bacteria mentioned, which present every variety of resisting power up to the almost incredible resistance of the spores of the hay and potato bacilli. The efficiency of the methods used for sterilizing surgical materials, as silk and catgut (Chapter IX., Part II.), should be tested; also, of the methods for disinfecting the hands; if possible, of the methods for disinfecting rooms, as well.

After some proficiency has been acquired, various pathogenic bacteria may be studied as the circumstances of the case require. Much judgment has to be used in allowing students to work with pathogenic bacteria. Anthrax, glanders, tetanus, cholera, bubonic plague, Malta fever, and diphtheria all have occurred in laboratory workers through accidental infection, sometimes with fatal results. Everything should be handled with forceps as far as possible, and the forceps sterilized in the flame before and after using. Particles of cotton fiber should not be allowed to fly off from the plugs. The various rules for the management of the platinum-wire, hanging-drop slides and sputum bottles, and for the handling of cultures and other infectious materials have already been given (pages 22, 23, 33 and 72 to 75). As the risks of infection from neglect of proper caution are obvious enough, it would seem, that it should be superfluous to warn students of the danger to themselves and others of infecting their hands and surroundings; but some who work in bacteriological laboratories become careless, just as do those who work with explosives. The most important precaution, perhaps, is observance of the rule that, while working in the laboratory, nothing should be put in the mouth. Cultures should never be left in improper places. Cultures of bacteria should be thoroughly sterilized before the tubes are cleaned. The writer is in the habit of having tubes and dishes containing pathogenic bacteria placed in the steam sterilizer at 100° C. for an hour on each of three days, and of having the

plugs removed and burned and the tubes filed with 5 per cent. carbolic acid between the second and third sterilizations. In taking these measures, the same kind of reasoning applies as that which induces engineers to give bridges from four to six times the strength they need to bear the greatest strain likely to be put upon them, or to make the boiler of a steam engine strong enough to bear six times the greatest pressure which it is expected that the steam contained in it will exert.

PART II.

CHAPTER I.

CLASSIFICATION; GENERAL MORPHOLOGY AND PHYSIOLOGY OF BACTERIA.

The relationships existing between bacteria and other kinds of organisms are not perfectly clear. It is quite generally conceded, however, that bacteria are plants. They show affinities with both the lower algæ and the lower fungi, but they have also some points of resemblance with certain of the protozoa. On account of their extreme smallness it is impossible to analyze the structure of the individual bacteria and to contrast the structure of one with that of another. The classification cannot therefore be established on morphological grounds chiefly, as is done with large animals or plants. We are obliged to rely also upon their growth with relation to the presence or absence of oxygen and to temperature, their behavior on culture-media, the appearances of the growths, and the production of certain substances with peculiar chemical reactions, when we wish to establish the points of difference between one species and another—all of which is extremely unsatisfactory and probably not perfectly trustworthy. The agglutination of a species of bacteria by blood-serum specific for the species (see Chapter VII., Part II.) has been used for purposes of identification. It is likely that forms which are now considered as different species are not really such in all cases, and also that different species may now be included under one heading as a single species. Notwithstanding the unsatisfactory condition

of the classification of bacteria, it must not be supposed that the species of bacteria are not permanent. For instance, it would be incorrect to imagine that the micrococci and spirilla become converted into species of bacilli, or for the bacilli of one species to be transmuted into those of another. This does not contradict the statement that we may frequently, through erroneous and imperfect information, be in the habit of including unlike species under one name, or of classifying mere varieties of one species as entirely different species. At present it is sufficient for practical purposes to divide bacteria into two great groups, the lower bacteria and the higher bacteria; and to subdivide the lower bacteria into: *micrococci*, spherical forms; *bacilli*, rod-shaped forms, one diameter being in excess of the others; *spirilla*, twisted like a corkscrew,

Staphylococci. Streptococci. Diplococci. Tetrads. Sarcinæ.

FIG. 39.

making long spirals or simply parts of spirals (comma-shaped forms).*

Recent investigations indicate that several species of bacteria often are closely related to one another, so as to form a well-marked group. Such a group is constituted by the bacillus of typhoid fever, bacillus coli communis and similar forms. The spirillum of cholera and other comma-shaped spirilla resembling it may be held to constitute another group. Still another is that containing the tubercle bacillus, smegma bacillus and other acid-proof bacilli.

The micrococci are subdivided into *staphylococci*, where

* Migula's system of classifying bacteria has found favor with many writers.

the spheres grow in clusters like a bunch of grapes; *streptococci*, where they are arranged in long rows or chains, like a string of beads; *diplococci*, or pairs of micrococci; *tetrads*, where the individual spheres are grouped in fours; *sarcinæ*, where they are grouped in eights, making the outline of a cube, resembling a bale or package tied with rope.

FIG. 40.—BACILLI OF VARIOUS FORMS.

The bacilli are not subdivided in this manner, although their forms vary considerably. The ends are sometimes square, sometimes round. Sometimes they are very short. Sometimes they grow in longer, thread-like forms, in which, however, the transverse markings which indicate the outlines of the individual bacilli can generally be seen, and which resemble a bamboo rod. Short, oval bacilli may look exceedingly like

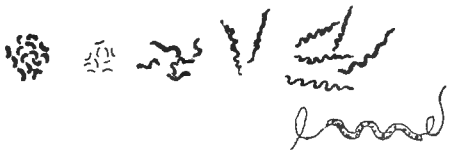

FIG. 41.—SPIRILLA OF VARIOUS FORMS.

micrococci. Bacilli with rounded extremities, placed end to end, look like strings of sausages. Under exceptional circumstances, branching forms of the bacilli of diphtheria, tuberculosis, glanders and bubonic plague and various other species have been encountered.*

* See Hill. *Journal of Medical Research.* Vol. VII. January, 1902. Loeb. *Ibid.* Vol. VIII. 1902.

The word "*bacterium*" was formerly used to designate short bacilli which generally formed no spores, while the word bacillus was restricted to the longer forms in which spore formation occurred. This use is no longer common, although not rarely the name bacterium is still given to a species—for instance, bacterium coli commune.

Spirilla present a very great variety of form. The short "*comma-shaped bacilli*" are only parts, at most, of spirals, although the microbes of cholera do sometimes form long spirals. On the other hand, there are among spirilla large and long sinuous figures which present most remarkable

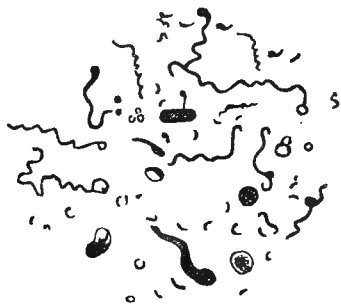

FIG. 42.—INVOLUTION FORMS OF THE SPIRILLUM OF CHOLERA.—(*Van Ermengem.*)

pictures under the microscope; for example, the spirillum of relapsing fever. Spirilla without very marked windings are sometimes called "*vibrios*"; and long, wavy forms with corkscrew-like windings "*spirochætæ*"; and only the rigidly spiral forms "*spirilla.*"

Besides the purely morphological classification already mentioned, bacteria are sometimes grouped according to certain physiological qualities. In general botany, saprophytes are plants that grow on decaying vegetable matter. In a bacteriological sense, *saprophytes* are bacteria which grow in external nature on dead organic matter, and *parasites* are bacteria

which exist upon the living tissues or fluids of any organism. Nearly synonymous with the above words are those which do not and those which do produce disease, or *non-pathogenic* and *pathogenic*. The adjectives *facultative*, or optional, and *obligate*, or strict, are used to qualify the above terms and many others.

Size.—Bacteria vary greatly in size. The micrococci are usually 1 μ or less in diameter. The short diameters of bacilli and spirilla also are less than 1 μ as a rule, while the length may be several micra. The anthrax bacillus (1.5 μ × 3 to 10 μ) and the spirillum of relapsing fever are the largest bacteria known to be pathogenic to man. To say that a micrococcus is 1 μ in diameter means that 25,000 end to end would make a line 1 inch long. It has been estimated that 1 milligram of a pure culture of the staphylococcus pyogenes aureus contains 8,000,000,000 micrococci.

There is good reason for believing that organisms exist which are too small to be visible with the most powerful microscopes. The nature of these organisms is not known, but it is not improbable that some of them are bacteria. (See pleuropneumonia of cattle, etc., Part II., Chapter V.)

In stained preparations the bodies of bacteria frequently seem to be homogeneous. On the other hand, they may exhibit certain spots which stain more intensely than others, the stained spots alternating with clear areas. The dark-staining granules may take a slightly different shade of color from the rest (metachromatic granules, Babes-Ernst bodies). Somewhat similar appearances may result from changes in the density of the protoplasm of bacteria, leaving vacuoles that do not stain (plasmolysis).

In old cultures bacteria are likely to show deformed and twisted outlines called *involution forms*. It is not uncommon for bacteria to be enclosed in a kind of envelope of some clear substance, which stains with difficulty or not at all,

called a *capsule.* The paired micrococci of pneumonia are enclosed in such capsules. The capsule is more likely to be demonstrated when the bacteria are obtained from the fluids derived from an animal's body than when they have been grown artificially in culture-media. A *zoöglœa* is a large mass of bacteria in a resting condition held together by a mucilaginous substance. The composition of bacteria varies considerably with different species. The basis appears to be proteid substance.

Vegetative Cells.—All the forms enumerated above are called *vegetative cells* in contradistinction to spores to be described later, and multiplication takes place by the direct division or *fission* of these cells. In the rod-shaped bacteria the fission is transverse. The formation of tetrads or sarcinæ from micrococci depends upon fission in two or three planes. Repeated fissions of micrococci in one plane result in the formation of streptococci. Micrococci that have recently divided are likely to be somewhat flattened on their opposing surfaces. Multiplication under favorable circumstances may take place at an exceedingly rapid rate. Bacilli have been observed to divide in twenty minutes. If division takes place once in an hour, the progeny of one organism at the end of twenty-four hours will be 16,777,216, *i. e.*, $(2 \times 1)^{24}$. The ordinary form of reproduction by fission is called *vegetative*, and bacteria that are multiplying in this manner are often spoken of as being in the vegetative condition.

FIG. 43.—BACTERIA WITH CAPSULES.

Spores.—Under certain circumstances the reproduction of bacteria takes place by means of the germination of bodies called *spores.* These appear in a typical form in the large bacilli, where, near the centers of the bacilli, highly refracting, shining spots may be seen which are found to stain less rapidly with the aniline dyes

than the rest of the bacilli. They are not to be confused with the unstained spots described as vacuoles. On account of their being formed from a part of the interior of the bacterium, such spores are called *endogenous*. These spores are found mostly in the bacilli, rarely in spirilla. They are what is meant when the word spore is used alone without qualification. The existence of another kind of spore, described as forming from the whole of the bacterium (called *arthrospore*), is doubtful. At all events, its significance is not at present understood. Spores develop generally, though not always, under adverse conditions of various kinds, as of temperature and of nutrition. They are more resistant to unfavorable influences of all sorts than are the fully developed bacteria. Spores resist drying, light, heat

FIG. 44.—BACTERIA WITH SPORES.

and chemical agents to a remarkable degree, at times. Spore formation is not a method of multiplication, since one spore when it germinates reproduces but one cell, and this cell then multiplies. So spore formation seems to be a means of preserving the organism under unfavorable environments, and not a process of reproduction in a strict sense.

Anthrax spores are said to have been found which could withstand steam for twelve minutes, 1–1000 mercuric chloride for nearly three days, or 5 per cent. carbolic acid for more than forty days. The greatest resistance is displayed by the spores of some of the saprophytic bacteria, particularly those of hay and potato, which are sometimes not destroyed by several hours of steaming; and bacteria which resisted 100° C. for sixteen hours

are said to have been obtained from the soil. When cultivated at a temperature as high as 42° C. the anthrax bacillus becomes incapable of forming spores. Spores themselves do not multiply, nor do they manifest any activity. Spores may be located at the center of the bacillus, or nearly at one end; in the latter case the end of the bacillus is likely to enlarge, making a form having the shape of a drumstick, as takes place with tetanus bacilli (Fig. 44). When a bacillus assumes a spindle shape on account of having the middle part bulged through the formation of a spore it is called a *clostridium*. With rare exceptions, a single bacillus contains but one spore. Under favorable conditions the spores germinate, as it is called, and develop to the adult form of the organism. This may be witnessed in hanging-drop preparations.

FIG. 45.—BACTERIA SHOWING FLAGELLA.

Motility.—Motility is rarely exhibited by micrococci; some bacilli possess it and some do not; while nearly all of the spirilla are motile. The phenomenon is observed in the hanging-drop. The degree of motility is variable, being sometimes slight and sometimes very active. When seen under a high power the little particles taken from a culture of a motile organism may look like a writhing mass of maggots or like tadpoles in a pool. The motility is most active in young cultures. The movement results from the vibration of little processes, or *flagella* (Fig. 45). Of these there may be one or several, placed singly or in groups, at the end, or scattered around the sides. They are extremely difficult to demonstrate except by special staining methods, which, furthermore, are quite uncertain of result.

After the flagella have been stained, the bacteria appear somewhat larger than when stained by the ordinary methods. The flagella upon the bacilli of typhoid fever are numerous and form a very striking picture.

Chemotaxis.—Motile bacteria possess the property of being attracted by certain substances (positive chemotaxis) and of being repelled by others (negative chemotaxis). Similar properties are widely distributed among living cells both animal and vegetable.

Conditions Favorable for the Growth of Bacteria.

Warmth.—Among the different kinds of bacteria forms exist which multiply at temperatures as low as 0° C., while there are species that multiply at 70° C. Bacteria which flourish at a very high temperature (maximum about 70° C.) are called *thermophilic*. The pathogenic bacteria usually flourish better at a point somewhere near the temperature of the human body. This is not necessarily the case with the non-pathogenic species. Ordinary water bacteria thrive better at ordinary temperatures.

Sternberg's method for determining the *thermal death-point* of a species of bacteria is to draw portions of a pure culture of the organism into capillary tubes with expanded ends, when the tubes are sealed in the flame. The tubes are supported upon a glass plate placed in a water-bath, whose temperature is indicated by a thermometer, while a uniform temperature is secured by stirring. The time of exposure is, as a rule, ten minutes. The tubes should be removed quickly to cold water. Their contents should afterward be inoculated into bouillon to determine whether or not the organisms have been killed. In the practical use of heat for sterilization or disinfection, the exact thermal death-point is greatly exceeded. The time of exposure is also longer than is absolutely necessary as determined by the results of the experiments.

Moisture is indispensable to the growth of bacteria, and drying causes the death of certain kinds, as, for instance, the spirillum of cholera, while others remain alive, but do not grow.

Food.—There are a few species of bacteria that contain chlorophyll, but it is wanting in most forms. On account of the absence of chlorophyll, bacteria require, as part of their food, organic compounds, such as sugar, as a source of carbon. They are unable, with very few exceptions, such as the nitrifying bacteria, to derive their carbon from the carbon dioxide of the atmosphere, or from inorganic carbon compounds. Although some species are able to obtain nitrogen from inorganic salts, most bacteria flourish best if organic substances containing nitrogen, like peptone and albumen, are furnished them as part of their food. The complicated, unstable, organic molecules with high potential energy are converted by them into simple and more stable compounds like carbon dioxide, ammonia and water, with the liberation of energy. These facts become manifest in connection with their important work in decomposition, putrefaction and fermentation. A culture-medium having a slightly alkaline or neutral reaction is favorable to most bacteria.

The prolonged artificial cultivation of bacteria may or may not modify their properties. The pathogenic bacteria are likely to undergo considerable modification both in the quality and luxuriance of their growth and the intensity of their pathogenic characters.

The growth of bacteria may eventually be hindered by the accumulation of the products of their own metabolism. Many bacteria refuse to grow on culture-media at all; at least the suitable artificial medium has not yet been found for them. Some species are extremely fastidious, and can only be propagated on particular nutrient substances. But bacteria show great adaptability, and, once they have been made to start, they can be further cultivated with less and less difficulty as a rule.

Relation to Oxygen.—Oxygen is indispensable to the

growth of some bacteria, *aërobes*. Its absence is equally indispensable to certain others, *anaërobes*. Others still are able to flourish either in the presence or absence of oxygen, facultative aërobes or anaërobes. The first-named varieties are sometimes called strict, or obligate aërobes or anaërobes.

Effects of Sunlight.—Direct sunlight kills the vegetative forms of bacteria more or less rapidly, and constitutes one of the most efficient among the natural methods of disinfection. Diffuse daylight acts much more slowly. Electric light acts like sunlight or daylight, the results being dependent on the intensity of the light. The violet part of the spectrum is most active.

The influence of *electricity* upon bacteria has not yet been fully studied. Apparently the destruction of bacteria reported as having been effected by electricity was the result of electrolysis of the medium.

It appears probable that *X-rays* do not produce important effects on bacteria, although further investigation of this subject is needed. The rays emitted by radium also require further study. Several observers report that radium rays have some germicidal power. The success which has attended the use of light rays, X-rays and radium rays, in the treatment of lupus and other diseases, is not necessarily to be explained as the result of bactericidal action of the rays.

CHAPTER II.

PRODUCTS OF THE GROWTH OF BACTERIA.

Phosphorescence.—Bacteria whose cultures exhibit phosphorescence have been found in the ocean and in fish.

Chromogenic Bacteria.—Many bacterial growths display brilliant coloring. The different species of sarcinæ are remarkable for forming highly-colored growths; some of them are rose-red, some orange-yellow, some lemon-yellow, and so on. The bacillus prodigiosus presents a brilliant red growth whose rapid development is said to have formed the basis for the so-called "Miracle of the Bleeding Host" (see page 5). The bacillus pyocyaneus in culture gives a brilliant green fluorescence and is responsible for the color of blue or green pus. Bacilli which exhibit a green fluorescence in cultures are common in water. In cultures on potato or agar the colors of the chromogenic forms are usually well shown. The pigment formed by the chromogenic bacteria is not produced in the cells themselves. These are colorless. The color is due to substances excreted by the cells, or formed from material in the culture-media.

Ferments or Enzymes.*—Many bacteria form ferments which have the power of dissolving proteid substances in a manner similar to trypsin. The liquefaction of gelatin is a familiar example of this process. The property of liquefying gelatin, or the failure to do so, is used in classifying bacteria and in determining the nature of unknown species.

Some bacteria, as the bacillus coli communis, form ferments

* Consult Buxton. Mycotic Enzymes. *American Medicine.* July 25, 1903.

which act like rennet in coagulating milk. Other bacteria are capable of forming sugar from starch. Others have the power of changing cane-sugar into glucose.

Bacteria which are able to decompose cellulose are found in the stomachs of ruminant animals. Although it is doubtful whether the products of cellulose decomposition have any nutritive value, the process is probably useful in effecting a subdivision of the coarse food, consisting of grass, hay and the like.

Some bacteria have the power of decomposing neutral fats into fatty acids and glycerin, after the manner of the fat-splitting ferment of the pancreatic juice.

The *end-products* which result from the growth of bacteria upon albuminous nutrient media are very numerous. They are complicated and not well understood. Among these end-products may be mentioned peptone, indol, skatol, phenol, leucin and tyrosin. Nearly related are the toxins (see Chapter VI.), which play an important part in the production of disease by pathogenic bacteria. In the decomposition of urine by bacteria the urea is converted into ammonium carbonate.

The *formation of indol* in cultures is an important peculiarity of certain bacteria, which may be tested as follows: The bacteria are cultivated in tubes of dextrose-free bouillon, or in Dunham's peptone solution, preferably the former; after twenty-four to forty-eight hours the test may be made. This consists in the addition of ten drops of concentrated sulphuric acid; the development of a rose-color indicates the presence of both indol and nitrites. If no rose-color forms, to another tube add, first 1 c.c. of a 0.01 per cent. solution of sodium nitrite, and then the sulphuric acid. The development of a rose-color indicates the formation of indol but not of nitrites. If there is no rose-color, no indol has been formed. The color appears usually in a few minutes, but it may only develop after a somewhat longer time. Control tests must be made upon tubes of the same bouillon but which have not been inoculated. The reaction may be hast-

ened by warming slightly. The value of this reaction will be understood when, to give one illustration, it is remembered that the bacillus coli communis produces indol and the bacillus of typhoid fever usually does not. The reaction depends upon the liberation of nitrous acid, which, with indol, forms a red color.

The change of organic substances into more stable ones does not cease with the compounds mentioned above. Certain bacteria of the soil which will be discussed further on are able to complete the conversion of ammonia into nitrous acid, leading to the formation of nitrites; and others still that of nitrites into nitric acid, which at once forms nitrates.

Formation of Acids.—In the course of their growth many bacteria produce acids, especially from substances containing sugar. The power of developing lactic acid is possessed by a large number of species. Acetic acid is another common by-product. Besides these, butyric acid, formic acid, propionic acid and many more are formed by different bacteria.

Development of Gas.—The evolution of gas from bacterial growths is of frequent occurrence. Carbon dioxide, hydrogen sulphide and nitrogen are among the better known gases that may be formed. The odors that arise from cultures and that are so characteristic of putrefactive processes depend upon the development of gases, or a mixture of gases, of considerable complexity. The bacillus aërogenes capsulatus leads sometimes to the formation of gas in the organs of the human cadaver within a short time after death. Theobald Smith introduced a valuable means of differentiating species of bacteria based upon their power of forming gas in media containing different sugars, or in their inability to do so. Bouillon containing 1 per cent. of dextrose (or lactose, etc.) is the culture-medium advised. The test is best conducted in a U-shaped tube, closed at one end, and at the other end provided with a bulb (Fig. 46). The tube is stoppered with cotton, sterilized by dry heat, afterward filled

with the bouillon, and sterilized by steam in the usual manner. After the last sterilization it should be tilted until the closed end is completely filled with the medium. After it has been inoculated with the species under consideration, any development of gas will be indicated by the collection of the gas at the closed end. The amount of gas formed may be estimated and its quality tested. To accomplish the latter fill the bulb with 2 per cent. solution of sodium hydroxide, close the outlet, and tilt the tube to allow the mixture to come in contact with the gas. After shaking, this causes the absorption of the carbon dioxide and diminution in the quantity of gas. The portions which remain may be mixed with air and ignited, when the presence of hydrogen and some of its compounds will be indicated by an explosion. (See The Detection of Bacillus coli communis in Water, Part IV.)

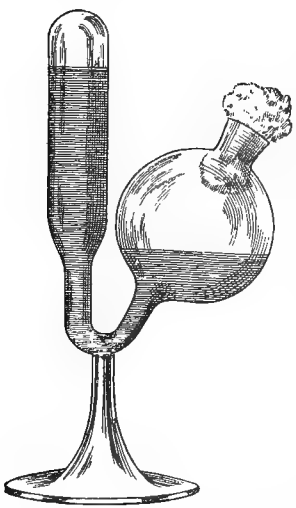

FIG. 46.—FERMENTATION-TUBE.

The development of gas may readily be tested by inoculating the bacteria by a deep puncture into agar containing 1 per cent. of dextrose or other sugars. The development of gas causes bubbles to form in the agar, often to the extent of splitting it, and sometimes forcing out the cotton plug (see Fig. 73).

The activities of bacteria which have just been enumerated are fundamental to the phenomena which go by the names of *fermentation* and *putrefaction*. These words have been defined differently at different times and by different writers, but in general both are used as names for the breaking up of complex organic compounds by micro-organisms with the formation of

simpler compounds. Fermentation refers especially to the formation of useful products like alcohol. The term putrefaction is employed chiefly for the breaking up of nitrogenous compounds with the development of foul-smelling gases. The term fermentation is also applied to the decomposition of complex substances through the influence of unorganized ferments or enzymes. The work of bacteria in decomposition is indispensable to the existence of the organic world as we find it. Green plants convert the stable compounds of nitrogen, the carbon dioxide of the atmosphere and water into the complex and unstable albumens and carbohydrates which serve as food for animals. Animals, on the other hand, convert these unstable and complex compounds back into simpler forms. The work of changing them back into the simple and stable condition, in which they serve as the food for plants, is performed by animal life in part only, and its completion is left to the activities of bacteria. It is the work of bacteria in this direction which we call decomposition. Without that work the existence of life upon the earth as we understand it would soon come to an end, and the dead and undecomposed bodies of living things and their products of all kinds would lie about unchanged, as they had fallen.

Bacterium termo is the name formerly given to a supposed species of bacteria which was credited with being the producer of putrefaction. The individuals were represented as being short rods, mostly going in pairs, and actively motile. The term has been abandoned since it appears to have included a number of different species.

CHAPTER III.

THE BACTERIA OF SOIL, AIR, WATER, AND OF MILK AND OTHER FOODS.

The Bacteria of the Soil.—Bacteria are present in the soil in enormous numbers—100,000 or more in 1 c.c. of virgin soil, according to Flügge. The depths to which they penetrate depend upon the character of the soil and the character of the life upon it, and whether or not it has been artificially disturbed, as by cultivation. In general, at a depth of 1.25 meters (about four feet) the number becomes very small, and a little deeper the soil is entirely sterile.

The bacilli of tetanus and malignant edema, and bacillus aërogenes capsulatus are present in the soil of many localities. According to Woodhead, certain savage tribes of Africa and the East Indies use as an arrow-poison soil that is capable of producing tetanus. The bacillus of anthrax may be found in soil which has been infected with this organism.

Most of the bacteria of the soil are harmless or useful saprophytes.* The *nitrifying bacteria* described by Winogradsky and by Jordan and Richards belong to the latter class. There occur in soil organisms which have the power of converting ammonia into nitrous acid which forms nitrites, and others which complete the change of nitrites into nitrates. Both varieties are widely distributed. These organisms will not grow on ordinary culture-media, and their cultivation presents great difficulties. Probably a good many bacteria have similar properties to some extent. The work done by nitrifying bacteria

* See Conn. Agricultural Bacteriology.

in making nitrates from sewage, manure and the like is indispensable to most plant life. Certain bacteria found in the soil are also concerned in the assimilation of free, atmospheric nitrogen, resulting in the addition of a valuable proportion of nitrogen compounds to the soil. This is spoken of as *nitrogen fixation.* Inasmuch as a large part of the excrementitious products of animals containing nitrogen are not retained in the soil, where they may be employed as food by plants, but are washed directly or indirectly into the sea by means of sewage and the rivers, it will be seen that the supply of nitrogen compounds might suffer gradual exhaustion. Furthermore, it has already been noticed (page 119) that one of the products of decomposition by bacteria is nitrogen, which is not available as food for animals or for most plants. These facts have met with practical recognition by agriculturists in the adoption of various methods of fertilizing the soil. It appears that the roots of peas, beans, clover, alfalfa and some other plants frequently present minute tubercles. The tubercles are pathological growths, caused by the development of microörganisms related to the bacteria. These organisms appear to have the power of assimilating atmospheric nitrogen and of converting it into nitrogen compounds. The same property probably belongs to some other bacteria of the soil. Experiments show that these observations may be destined to be of great value to the farmer.*

The bacteria of the soil may easily be studied in plate-cultures made from small portions of soil collected with the necessary precautions to avoid contamination, or plate-cultures may be made from sterilized water with which a portion of the soil has been properly mixed. Anaërobic bacteria must be cultivated by the special methods adapted to them.

* For simple experiments to illustrate these phenomena see Buxton. *Journal of Applied Microscopy.* September, 1902. For practical application to agriculture consult G. Moore. U. S. Dept. Agriculture. Bureau of Plant Industry, Bulletin No. 71. Jan. 23, 1905.

Bacteria of the Air.—The bacteria of the air will be found for the most part clinging to solid particles in suspension in the shape of dust. As has already been stated, bacteria cannot be blown from moist surfaces, they are not removed by currents of air. Conditions of dryness and wind tend to increase the number of microörganisms in the air. They are fewer after a fall of rain or snow, and the number is smaller in winter than in summer. The air of cities contains more bacteria than that of the country. The atmosphere over the sea and at the tops of high mountains is nearly or wholly free from bacteria. The bacteria which do occur in the air are seldom pathogenic. Their character depends upon the character of the dust. It is obvious that dust which consists in part of the dried, pulverized expectoration of cases of pulmonary tuberculosis may contain tubercle bacilli. Anthrax of the lungs sometimes arises in men who handle the wool of sheep that were infected with anthrax (wool-sorter's disease), and is due to the inhalation of anthrax spores attached to the wool. The atmosphere in the immediate vicinity of cases of the exanthematous fevers is liable to contain the organisms, whatever they may be, that cause these diseases.

In a rough way one may obtain some knowledge of the character of the organisms in the air of a given locality by removing the cover of a Petri dish containing sterilized gelatin or agar or thin slices of boiled potato for a few minutes, replacing it, and allowing the organisms to develop. In most cases a large proportion of the growth that appears will be moulds. Yeasts are also common, and among the bacteria the micrococci are abundant. Chromogenic varieties are likely to be present.

A few studies of this character will show that the number of organisms that are present depends chiefly upon whether the air is quiet or has recently been disturbed by draughts, gusts of wind, or sweeping. These facts are of fundamental importance in laboratory work, where plate-cultures are being

studied, if we wish to avoid contamination of the plates. Among various devices that have been proposed for the accurate study of the organisms of the air, the Sedgwick-Tucker aërobioscope is the simplest and most accurate. It consists of a glass tube, one end of which is drawn out so as to be smaller than the other. The small end contains a quantity of fine, granulated sugar; both ends are plugged with cotton, and the instrument is sterilized. After removing the cotton a definite quantity of air is to be aspirated through the large end, which may be done by means of a suction-pump applied to the other end, or by siphoning water out of a bottle the upper part of which is connected with the end of the aërobioscope by means of a rubber tube. The sugar acts as a filter and sifts out of the air the microörganisms which are contained in it. Liquefied gelatin or

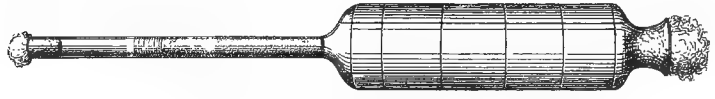

FIG. 47.—SEDGWICK-TUCKER AËROBIOSCOPE.

agar is introduced into the large end of the instrument by means of a bent funnel; and, after replacing the cotton, it is mixed with the sugar which dissolves. The culture-medium is spread around the inside of the larger portion of the tube after the manner of an Esmarch roll-tube. The bacteria which are filtered out by the sugar develop as so many colonies upon the solidified medium.

Bacteria of Water and of Ice.—The water of rivers, lakes and the ocean always contains bacteria. The number of organisms varies greatly in different places and under different conditions. The number of different species found in water is also very large. Ground-water * contains few or no bacteria

* Ground-water is the water which—originally derived from rain or snow—sinks through superficial porous strata, like gravel, and collects on some underlying, impervious bed of clay or rock.

under normal conditions, and is therefore suitable for a source of water-supply, when a sufficient amount is available. The possibility of contamination of the ground-water from unusual or abnormal conditions should always be eliminated before it is taken for drinking-water. Numerous epidemics of typhoid fever have been traced to contamination of wells. The location of wells with reference to privy-vaults and other possible sources of contamination should be chosen with the greatest care.

The ordinary bacteria of water* are harmless, as far as is known. Bad odors and tastes in drinking water that is not polluted with putrid material are usually due to minute green plants (algæ).† The diseases most commonly disseminated by water are typhoid fever and Asiatic cholera, and probably also dysentery. The results of experiments testing the length of time which the cholera spirillum and the typhoid bacillus may persist in water are conflicting. Many epidemics of cholera and typhoid have been traced to water polluted with the discharges from cases of these diseases.

By *self-purification* of water is meant the removal through natural processes of contaminating organisms such as might occur from the discharge of sewage into it. It depends upon the sedimentation of the contaminating material, in the form of mud, upon the growth of the ordinary water-plants and protozoa, upon the exhaustion of the food supply by the growth of bacteria themselves, upon the destructive influence of direct sunlight, and the dilution of the matter added with a large volume of water.‡ It is not usually to be relied upon as a means of freeing the water-supply from pathogenic bacteria.

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* See Fuller and Johnson. The Classification of Water Bacteria. *Journal of Experimental Medicine.* Vol. IV. p. 609. Jordan. *Journal of Hygiene.* Vol. III. Jan., 1903.

† G. T. Moore. Contamination of Water Supplies by Algæ. Yearbook U. S. Department of Agriculture. 1902.

‡ See Jordan. *Journal of Experimental Medicine.* Vol. V., p. 271.

Storage of Water.—When water is kept in large reservoirs, the solid particles in it, including bacteria, tend to fall to the bottom. The number of bacteria in a water-supply may be considerably reduced in this way.

Filtration.—Filtration on a large scale has been more commonly in use in the cities of Europe than elsewhere, until lately. But filtration-plants now exist in several cities of the United States. By this method 98 per cent. to 99 per cent. of the bacteria in water may be removed.

*Slow Sand Filtration.**—The filter consists of successive layers of stones, coarse and fine gravel. The uppermost layers are of fine sand. The whole filter is from 1 to 2 meters thick. The sand should be 60 cm. to 1.2 meters in thickness. The upper layers may be removed from time to time, the remainder not becoming less than 30 cm. in thickness. The first water coming from the filter is discarded. The actual filtration is done largely by the slimy sediment which collects on the surface of the layer of fine sand. The filter-beds may be several acres in extent, and in cold climates should be protected by arches of brick or stone. They require renewal occasionally. This kind of filtration has come largely into use since the cholera epidemic of 1892–93, and it appears to be very effective. It is often advisable to use storage basins in connection with sand filtration, to permit of settling of part of the solid matter before filtration.

Mechanical Filtration.—This method of filtration is also called the American system. It is more rapid than the preceding method and does not require a large area for filter beds. Although sand is required also, filtration is accomplished by a jelly-like layer of aluminum hydroxide. This product is formed by adding to the water a small quantity of aluminum sulphate

* For a full discussion see *Journal American Medical Association.* Oct. 3, to 31, 1903.

or salt of alum. The carbonates in the water decompose the aluminum and produce aluminum hydroxide. It precipitates as a white, flocculent deposit, entangling solid particles, including bacteria, as coffee is cleared with white of egg. Only a trace of aluminum should appear in the water. This method of filtration has not been tested so extensively as slow sand filtration, but seems likely to prove efficient. With water poor in carbonates, these may have to be added.*

Various methods for the purification of water by means of chemicals have been proposed. The use of ozone for this purpose has met with considerable favor.†

The filtration of water on a small scale, as is ordinarily done for domestic purposes, is generally entirely useless. The so-called Pasteur filter of unglazed porcelain is effective if it is properly constructed and if the filter-tubes are sterilized by heat every few days—conditions which are seldom complied with. Distillation of water and boiling are the most practical methods for sterilizing drinking-water.

Collection of Samples.—For bacteriological examination samples from the water-supply of a city may be drawn from the faucet, but the water should first be allowed to run for half an hour or longer. From other sources the supply should be collected in sterilized tubes or bottles, taking care to avoid contamination. Sternberg bulbs (see Fig. 38) will be found useful for small samples. These samples should be examined as quickly as possible, for the water bacteria increase rapidly in number after the samples have been collected. When transportation to some distance is unavoidable the samples should be packed in ice.

The **number of bacteria** may be determined by making plates of a definite quantity of the water with gelatin or agar.

* See Fuller. *Journal American Medical Association.* Oct. 31, 1903.

† Consult Rosenau. Disinfection and Disinfectants. 1902.

The amount examined ordinarily is 1 c.c. When the number of bacteria is very large, a smaller quantity must be taken, and it may be necessary to dilute the sample ten times or more with sterilized water. The amount should be measured with a sterilized, graduated pipette. The water is mixed with melted gelatin or agar in a tube which has been allowed to cool after melting. After thorough mixing, remove the plug, burn the edge of the tube in the flame, hold in a nearly horizontal position until cool and pour into a sterilized Petri dish. The number of colonies may be counted on the third or fourth day; the later the better, as some forms develop slowly and may not present visible colonies for several days; but the plates are often spoiled after three or four days by the profuse surface growths of certain forms or by the rapid liquefaction of gelatin, if that be used, by other forms. The number of colonies that develop is supposed to represent the number of individual bacteria contained in the quantity measured. That will probably not always be the case, however, as colonies may develop from a clump of bacteria which have not been separated from one another by the mixing process. Abbott has shown that the number of colonies is usually larger on gelatin plates than upon agar plates, and at the room temperature than in the incubator. This observation illustrates the fact that there are doubtless many kinds of bacteria that do not find favorable conditions for development on ordinary culture-media. The reaction of the medium has an important influence upon the development of these water bacteria in plate-cultures.

When the number of colonies is small, there is no difficulty in counting them as they appear in the ordinary Petri dish. When the number is large some kind of mechanical device may be used to assist counting. The Wolffhügel plate is a large square of glass resting in a wooden frame painted black. The glass plate is ruled in squares. It was designed particularly with reference to the form of plate-cultures first made by Koch.

The Petri dish, however, may be placed upon the glass plate and the cross lines be used to assist in counting. Lafar, Pakes and Jeffer recommend a surface painted black, ruled with white lines which represent the radii of a circle, which may be still further subdivided by other lines. Many find counting easier when a black surface divided into squares is employed. An ordinary card with a smooth black surface divided into squares by white lines may be placed under a Petri dish and will be found to serve very well.* For the mere examination of the colonies no better surface can be devised than the ferrotype plate used by photographers. The examination of the colonies will be easier if a small hand-lens be used. Care must be taken not to mistake air-bubbles or particles of dirt for colonies of bacteria.

In any case, if possible, all the colonies in the plate should be counted. The number contained within several squares may be counted and the average taken; knowing the size of the squares and the area of the plate, the number contained in the whole plate may be calculated. Such estimations, however, are likely to give results very wide of the truth.

The plating may be done by rolling the medium after the manner of Esmarch. When the number of colonies is not large this may serve very well. Counting may be assisted by drawing lines with ink on the outer surface of the test-tube.

It has been said that a water-supply containing no more than 500 bacteria per cubic centimeter is to be regarded as safe, one having between 500 and 1000 is to be looked upon with suspicion, and that where there are more than 1000 to the cubic centimeter the water is unfit for drinking purposes. It is obvious, however, that the character of the bacteria is of prime importance; that pathogenic organisms may occasionally be present, even when the number of bacteria to the cubic centimeter is small. But knowing the number usually found

* Specially ruled cards will be found after the index at the back of the book.

in a good water-supply, any sudden variation above that number is to be looked upon with suspicion as indicating a possible surface contamination.

The bacteriological examination should always be accompanied by a chemical examination, and by an inspection of the surroundings. A large number is to be expected when the water has been subjected to unusual agitation from winds or currents which stir up the bacteria which have settled.

The detection of pathogenic bacteria in water * involves great difficulties, and our knowledge in this direction is very meagre. Koch and several others have reported finding the spirillum of Asiatic cholera in water. The examination of water-supplies for this organism has disclosed the fact that bacteria resembling the organism of cholera in many respects are not uncommon in water. This adds to the difficulty of detecting the cholera germ in water.

The bacillus of typhoid fever has many times been described as occurring in water-supplies suspected of being contaminated with the excreta of cases of the disease. The interpretation of these observations is at present doubtful.† It is now known that several forms of bacteria exist which closely resemble the bacillus of typhoid fever, and which make its recognition in an unknown specimen very difficult.‡

It will at once be appreciated that the number of cholera and typhoid organisms necessary to contaminate a considerable body of water, and sufficient to cause an outbreak of the disease among some of the people drinking the water, may still be so small that many different cubic centimeters of the water might be studied before a single one of the specific organisms would be encountered. Anyone who has examined plates

* See also articles in Part IV. on the bacillus of typhoid fever, bacillus coli communis and spirillum of cholera.

† Consult editorial. *Journal American Medical Association.* Dec. 5, 1903.

‡ For methods of detection see under Typhoid Bacillus, p. 301.

made from samples of water will recognize the difficulty of detecting one or a few colonies of the bacteria of cholera or typhoid fever among a hundred or more colonies of ordinary water-bacteria. The existence of contamination with animal excreta might, however, be indicated by finding the bacillus coli communis, whose detection offers a greater prospect of success. It is not certain just how much importance is to be attributed to the presence of small numbers of the colon bacillus in water.* Until our knowledge is more complete any suspicious water should be discarded.

At present investigators seem to agree that if, using several samples of a water each 1 c.c. in volume, colon bacilli are found in a majority of the samples the water is probably polluted; if the colon bacillus is only found when larger volumes of water are examined, the results are suspicious though less significant. Some investigators hold that the presence of streptococci in water is indicative of pollution.†

Certain devices have been adopted to hasten the development of the desired bacteria and to retard that of the ordinary water-bacteria. Among these may be mentioned the influence of the heat of the incubator, which will hasten the growth of organisms derived from the human body, and which retards the growth of water-bacteria. Another is the addition of a solution of peptone to a large quantity of the water to be examined with a view to assisting the development of the desired bacteria by furnishing them suitable food for growth. In another method (Parietti's) small quantities of carbolic acid are added to bouillon and mixed with the water, with a view to retarding the development of all except typhoid ‡ and colon bacilli. Suspected bacteria may be tested by inoculation into

* Jordan. *Journal of Hygiene.* Vol. I. 1901. Savage. *Journal of Hygiene.* Vol. II. 1902. Winslow and Hunnewell. *Journal Medical Research.* Vol. VIII. 1902.

† Prescott and Baker. *Journal of Infectious Diseases.* I. 193.

‡ Prescott. Report of American Public Health Association. Vol. XXIX, 356. Clark and Gage. *Ibid.* 386. Bissell. *Ibid.* 360.

animals; the possession of pathogenic properties creates a probability in favor of their having come from some contamination with animal excreta.*

Ice.—The bacteriological examination of ice differs in no respect from that of water. Although development may be arrested, the vitality of bacteria is not necessarily impaired by freezing. Prudden found the bacillus of typhoid fever alive in ice after more than one hundred days. However, Sedgwick and Winslow have stated that when typhoid bacilli are frozen in water the majority of them are destroyed.† Nevertheless, it is safest to have the source from which ice is taken as carefully scrutinized as that of the water-supply, especially in view of the universal habit of cooling water in the summer time by adding ice directly to the water. It is better to cool water and articles of food by surrounding the vessels containing them with ice.

Bacteria of Milk and Other Foods.‡—Of the different food substances, milk is probably the most important from a bacteriological point of view. In the first place, most other foods are cooked before eating. Furthermore, cow's milk constitutes the principal food of young infants, who are highly susceptible to certain bacteria, and to substances in the milk itself, after it has undergone certain alterations due to bacteria. The milk of the healthy cow as it is secreted in the mammary gland is sterile; however, after milking the cow a little milk generally remains in the milk-ducts and in the lower part of the teat in which numerous bacteria will have developed before

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* Consult Vaughan. *Journal American Medical Association.* April 9, 1904. For special methods of detecting the Bacillus coli communis see under this bacillus, page 310.

† Clark. Bacterial Purification of Water by Freezing. Reports American Public Health Association. Vol. XXVII. See also Hutchings and Wheeler. *American Journal Medical Sciences.* Vol. CXXVI., p. 680.

‡ See Conn. Bacteria in Milk and its Products. 1903. Russell. Dairy Bacteriology.

the next milking-time.* The first milk obtained at a milking should therefore be discarded, as it may contain an excessive number of bacteria.

Contamination with bacteria may occur from the outer surface of the udder of the cow, the hands of the milker or dirty pails, or through agitation of the air of the stable, and in other ways readily conceived of. Bacillus coli communis is often found in milk. Excluding the tubercle bacillus, the organisms which contaminate milk are pathogenic only in exceptional cases. Occasionally typhoid fever, cholera, probably scarlet fever, and possibly diphtheria and other diseases are disseminated by means of contaminated milk. In the case of typhoid fever, the milk cans may have been washed with polluted water; after the cans were filled, a few typhoid bacilli left in drops of water in the cans may multiply enormously. Streptococci have been found quite frequently in the milk sold in cities.† The mixture with the milk of non-pathogenic organisms from the air, and their growth, may induce changes in it which render it unfit for consumption, and even poisonous. These alterations may be evident to the senses, as the ordinary lactic acid fermentation (souring of milk), or they may not. The character of the alterations doubtless varies much with the temperature and with the character of the contaminating bacteria. Summer temperatures of course favor decomposition and fermentation. Specialists in children's diseases attribute to alterations in milk with the formation of poisonous substances a preëminent influence in the production of the intestinal disorders of infancy so common in the summer.

Poisoning with milk, ice-cream or cheese is not rare, as is well known. There are many records of whole companies

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* See Harrison and Cumming. The Bacterial Flora of Freshly Drawn Milk. *Journal of Applied Microscopy.* November, 1902.

† See Reed and Ward. The Significance of the Presence of Streptococci in Market Milk. *American Medicine.* February 14, 1903.

of individuals having been taken violently ill after having eaten one of these foods from the same source of supply. The symptoms in such cases resemble those produced by irritant mineral poisons such as arsenic: nausea and vomiting, vertigo, dryness of the mouth, sense of burning and constriction in the throat, difficulty in swallowing, cramps and griping pain in the bowels, constipation or diarrhea, general prostration or even collapse. Vaughan isolated from poisonous cheese a ptomaine which he called tyrotoxicon. It appears, however, that other toxins may be present in cheese, and that tyrotoxicon is a somewhat rare poison. Vaughan believes that bacteria of the colon group play an important part in producing poisons in milk and cheese.

To prevent the alteration by bacteria of milk intended to be the food of infants, the practice of sterilizing milk has been largely in vogue. Unfortunately, during sterilization the milk undergoes some kind of alteration which makes it disagree with certain infants. Furthermore, among the organisms which would be likely to contaminate milk bacteria such as the bacilli of hay and potato, whose spores are so excessively resistant, would be prominent, and they are not killed by any process to which the milk intended for an infant's consumption could possibly be subjected in the household. Least of all can sterilization be relied on to purify milk in which bacterial poisons are already formed.

The investigations of Park and Holt* show that in New York City the number of bacteria in milk is much smaller in winter than in summer, and has little effect on the health of infants during cold weather; but that in warm weather with milk of average quality the infants who received sterilized milk throve on the average much better than those who received raw milk.

**Archives of Pediatrics.* December, 1903.

The process called pasteurization is designed, not to sterilize the milk completely, but to destroy the vegetative forms of bacteria, and to destroy the ordinary pathogenic bacteria with which the milk might possibly be contaminated.* The milk is subjected to a temperature of only about 70° to 75° C. This temperature is less likely to produce alteration in the milk than sterilization by steam at 100° C. According to Freeman, milk which had been pasteurized at 75° C. and distributed among the poor people of New York City, whose homes were not supplied with ice, usually kept very well even in the summer time (see p. 55).

The number of bacteria in milk may be reduced considerably by the use of the centrifuge (separator).

It has been undertaken recently to do away so far as possible with the contamination usually occurring in the barnyard and stable by the use of extraordinary measures to keep the cows, and especially their udders, clean; also the hands of the milker and the milk-pails; and by sprinkling the floor of the milk-room to prevent dust.† The milk should be transported to the city on ice. Milk which has been collected in this manner is furnished in several cities in the United States. The cattle from which the milk is derived are regularly inspected by veterinary surgeons as well as subjected periodically to the tuberculin test. The surroundings and drainage of the stables are investigated by physicians and sanitary engineers. The milk is also regularly analyzed by a chemist. It has been found possible to reduce the number of bacteria in milk to a few thousand per cubic centimeter. This milk is of course sold at a considerably higher price than ordinary milk.

The number of bacteria which occur in samples of milk

* Theobald Smith. The Thermal Death-point of Tubercle Bacilli in Milk, etc. *Journal of Experimental Medicine.* Vol. IV., p. 217.

† W. H. Park. *Journal of Hygiene.* Vol. I. 1901.

varies greatly. In ordinary milk as furnished by milkmen the number of bacteria to the cubic centimeter is usually many thousands up to many millions; grocer's milk may contain hundreds of thousands or millions of bacteria to the cubic centimeter; frequently figures are reached which are beyond computation.

Human milk often contains the staphylococcus epidermidis albus, and not seldom the staphylococcus pyogenes aureus, under normal conditions.

Of the different pathogenic bacteria liable to furnish a source of danger in milk, the most important is the bacillus tuberculosis. Tuberculosis is a disease to which cattle are exceedingly prone. There is good reason to believe that infants acquire tuberculosis through taking as food the milk of tuberculous cows, although the danger from this source has probably been overestimated. The milk of tuberculous cows may obtain tubercle bacilli when there is no tuberculous disease of the udder.* The frequency of tuberculosis among milch cows sometimes becomes as high as 25 per cent., or even more. Butter derived from the milk of such cows may contain tubercle bacilli. The proper manner for the States to deal with this problem, for it is one that doubtless will fall to the individual States, has not yet been determined. The cost of killing such a large number of valuable cows would be very great. Furthermore, it is by no means certain that this procedure would eradicate the disease. The flesh of cattle also is capable of transmitting tuberculosis, but is a less serious source of danger when beef is thoroughly cooked.

"Ripening" of cream and cheese is due to the growth of bacteria which produce agreeable flavors in the butter and

* Mohler. Infectiveness of Milk of Cows which have Reacted to the Tuberculin Test. U. S. Dept. Agriculture, Bureau Animal Industry, Bull. No. 44. 1903.

cheese. Moulds are also important in the ripening of some kinds of cheese.†

In examining milk for bacteria the number may be estimated by precisely the same technique as is used for the estimation of bacteria in water, except that the milk must be diluted; otherwise the plates are rendered opaque by the fat. It may be diluted one hundred times with sterilized water; when the number of bacteria is very great a second dilution may be required. Estimations based upon such high dilutions can only be approximate. The quantity taken for examination may be 0.1 to 1 c.c. Plates should be made immediately upon collection of the sample. If the milk stands for a few hours at the room temperature in the laboratory, the number of bacteria becomes enormously increased.

The detection of a particular kind of pathogenic bacteria in milk or butter involves very great difficulties. Staining of bacteria in milk may be done by the usual methods, but the results are rendered unsatisfactory by the oil in the milk. The demonstration of tubercle bacilli by staining methods is likely to involve many difficulties. In this connection it is necessary to remember the group of bacilli which resemble the tubercle bacillus in resisting decolorization with acids after staining. (See p. 32.) The procedure of injecting milk into guinea-pigs has been resorted to largely, but the results are only obtained after the lapse of weeks, when the development of tuberculosis in the guinea-pigs indicate that the milk was tuberculous, provided that control guinea-pigs remain healthy. Furthermore, the other acid-proof bacilli which may occur in milk or butter are capable of producing nodules resembling tubercles.† (See .Bacillus tuberculosis, Part IV.) The most satisfactory plan is to apply the tuberculin test to the cow from which the milk is derived.

Among the other articles of food, those are to be most care-

* Conn. Agricultural Bacteriology.

† Rabinowitsch. *Zeitschrift f. Hygiene.* Bd. XXXVII., p. 439.

fully scrutinized which are to be eaten after little or no cooking, particularly salads, green vegetables, fruits, and the like. Under exceptional circumstances they may become agents for conveying infectious diseases. Conn showed that there was good reason for attributing an epidemic of typhoid fever among students at Middletown, Connecticut, to raw oysters. After having been collected from the oyster-beds, these oysters were placed in a small stream to fatten, where they were exposed to contamination from a sewer. Into this sewer the discharges of a case of typhoid fever were found to have been running at the time when the oysters were fattening. An epidemic at Atlantic City, New Jersey, in 1902, was traced to nearly similar causes and conditions.*

The ordinary processes for curing and salting meat cannot be relied upon to destroy pathogenic bacteria.

Cases of poisoning by eating oysters, fish, meat in the form of sausage or canned meat, and other articles of food are not rare. These cases belong to the same class as those poisoned by milk and cheese already mentioned. They are due to products of bacterial decomposition. Such affections are quite commonly called ptomaine poisoning, although the poisons are not ptomaines in most cases. Probably a number of bacteria exist which are capable of affecting changes in meat and other foods either before or after ingestion. Among these are an anaërobic bacillus described by Van Ermengem (B. botulinus), various members of the groups represented by B. proteus and B. coli communis (including paracolon bacilli), and the bacillus enteritidis of Gaertner. In the case of B. enteritidis a genuine infection of the patient and gastro-enteritis may occur.†

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* *Philadelphia Medical Journal.* November 1, 1902.

† See Vaughan and Novy. The Cellular Toxins. 1902. Ohlmacher. Food-Intoxication from Oatmeal. *Journal of Medical Research.* Vol. VII., p. 420. Galeotti and Zardo. *Centralblatt für Bakteriologie.* Vol. XXXI. 1902. Orig. p. 593.

CHAPTER IV.

THE BACTERIA OF THE NORMAL HUMAN BODY.

The numerous solid tissues and organs of the human body, the fluids circulating in the interior like the blood and lymph, and the cavities that have no connection with the outer world, are entirely free from bacteria.* So also the maxillary, ethmoidal and frontal sinuses, middle ear,† urinary bladder, uterus and Fallopian tubes, and to a less extent the lungs ‡ and gall-bladder,§ although having external connections, are usually sterile when in a healthy condition. When bacteria do enter the tissues from any of the surfaces their progress is checked by means of the activities of the cells or fluids of the body, and if they succeed in penetrating to any considerable distance their advance is usually arrested by the nearest group of lymph-nodes, which appear to be important safeguards for preventing the dissemination of bacteria throughout the body. As a rule, the secretions of the mucous membranes are inimical to bacteria.

The skin,‖ as might be expected, is liable to have upon it

* This view is not upheld by the experiments of Ford, who found small numbers of bacteria in the normal organs of rabbits, cats and dogs in the majority of those examined. The species of bacteria obtained were mostly common saprophytes, and to some extent constant in the same kind of animal. *Journal of Hygiene.* Vol. I. 1901.

† Calamida and Bertarelli. *Centralblatt für Bakteriologie.* Vol. XXXII. 1902. Orig. p. 428. Törne. *Ibidem.* XXXIII. 1903. p. 250. Hasslauer. *Ibidem.* Referate. XXXII, p. 174. An examination of these articles will show that investigators disagree somewhat, with regard to the sterility of these cavities.

‡ See Wadsworth. *American Journal Medical Sciences.* May, 1904.

§ See Bacteriology of the Gall-bladder and its Ducts. *American Journal Medical Sciences.* Vol. CXXIII., p. 372.

‖ Sabouraud. La Peau Humanie, etc. *Bulletin de l' Institut Pasteur.* II. 1904. Pages 233, 282.

numerous bacteria, especially micrococci, and moulds. The staphylococcus pyogenes aureus, the streptococcus pyogenes, the bacillus pyocyaneus and the bacillus coli communis sometimes occur on the skin. According to Welch, it always contains the staphylococcus epidermidis albus, which may be a form of the staphylococcus pyogenes albus. This organism is of some importance to surgeons on account of its relation to the cleansing of the skin before operations. It seems impossible, by any amount of cleaning, to dislodge all of the germs in the skin, especially those under the nails.

The bacteria of the exposed mucous membranes like the conjunctiva* and the nasal cavity† and the mouth cavity naturally fluctuate both in quantity and quality; they consist, in fact, of those which happen to fall upon the surface or are drawn in from the external air.

In the mouth, however, there is a certain group of organisms more or less characteristic of it, many of which have not been successfully cultivated. These have been thoroughly studied by Miller, to whose works students are referred.‡

Several species of spirilla have been discovered in the mouth and are found along the margins of the gums. The leptothrix buccalis, and related organisms which have a long, ribbon-like form, also occur in the mouth. The micrococcus lanceolatus (or pneumococcus) is present in many human mouths. In 15 to 20 per cent. of human mouths this organism is sufficiently virulent to produce fatal septicemia when inoculated into susceptible animals. Pyogenic bacteria, especially streptococci, occur frequently, although not regularly, in the mouth. Strep-

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* Randolph, Pusey, Gifford. *Journal American Medical Association.* Oct. 3, 1903.

† Hasslauer. Die Bakterienflora der gesunden und kranken Nasenschleimhaut. *Centralblatt für Bakteriologie.* Vol. XXXIII. 1902. Orig. p. 47.

‡ Miller. Microörganisms of the Mouth. For a recent review on the bacteria of the mouth, see Madzar. *Centralblatt für Bakteriologie.* Vol. XXXI. Ref. p. 489. Vol. XXXII, p. 609.

tococci very commonly occur on the tonsils. Putrefactive bacteria acting on particles of food about the teeth produce the bad odor from the mouths of persons of careless habits. According to Miller, bacteria play an important part in the production of dental caries. Certain of the bacteria of the mouth produce fermentation in the vicinity of the teeth with the formation of acids, which dissolve the calcium salts of the teeth. The softening and destruction of the decalcified matrix is then accomplished by other and liquefying forms.

The expired air coming from the mouth and nose, contrary to the popular notion, is free from bacteria, excepting those which become forcibly detached, as by efforts of sneezing and coughing.

Among the other exposed mucous surfaces, the urinary meatus and the vagina may be included. The urinary meatus and at least part of the urethra will be found to contain bacteria, which, in health, should be non-pathogenic, although interest attaches to the fact that diplococci have been described which behaved with stains in the same manner as the gonococcus (pseudo-gonococci).

There has been much dispute as to whether or not the pyogenic bacteria occur in the vagina normally. It is probable that the healthy vagina is in most cases free from the pyogenic bacteria; although bacteria of some sort are always present, and the pyogenic bacteria may exceptionally be found there in health. The normal secretion of the vagina has a bactericidal influence which may be attributed in part to its acidity. The upper part of the normal cervix uteri is sterile, while bacteria are present in the lower part.

According to Döderlein, the properties of the vaginal secretion are due to bacilli which very commonly occur in it. The secretion is most abundant and important during pregnancy.*

*J. W. Williams. Obstetrics. A Text-book, etc. 1903. Pp. 34, 773–775. Wadsworth. *American Journal of Obstetrics.* Vol. XLIII. 1901.

The smegma of the external genitals contains numerous bacteria, among which are frequently found bacilli which retain their color after treatment with acids in the Gabbett method for staining tubercle bacilli. It is uncertain whether these bacilli form a special group of organisms by themselves, having as one of their properties the power of retaining the stain after acids, or whether they are bacilli of no particular sort, which resist acids after staining owing to the oily material with which they have been impregnated in this peculiar secretion. These organisms must be taken into account in staining for tubercle bacilli, urine or other secretions which might accidentally contain particles of smegma. Usually the employment of alcohol after the acid will remove the color from the smegma bacilli (Hueppe). Sometimes smegma bacilli are as resistant as tubercle bacilli to decolorizing agents (Welch); see page 33. Similar acid-proof bacilli occur about the genitals of the domestic animals.*

The bacteria of the stomach and intestines are of great interest and importance. The alimentary tract of new-born infants and the meconium are sterile. In from four to eighteen hours organisms begin to appear. They may enter either from the mouth or the anus. There seems to be no constancy in the nature of the forms which are found at first, but their character depends upon the surroundings.

The bacterial inhabitants of the stomach are less constant than we shall find those of the intestines to be. Under normal circumstances they seem to be those introduced from the mouth. Different investigators, at all events, have met with quite different species. It appears that the hydrochloric acid (about 2 parts per thousand) present in the gastric juice at the height of digestion possesses decided germicidal properties. This germicidal power exercises a restraining influence upon fer-

* Cowie. *Journal Experimental Medicine.* Vol. V., p. 205.

mentation due to bacteria, and probably serves as a safeguard against the introduction of pathogenic germs into the intestines. That is particularly important in the case of the spirillum of cholera, which is excessively sensitive to the action of acids. Nevertheless many bacteria are able to reach the intestines uninjured, as the acidity of the gastric juice does not reach its height until some hours after eating. Such bacteria will be those which are most resistant and those which form spores. In the intervals when hydrochloric acid is absent from the stomach, lactic acid appears. It is formed from carbohydrates by a large number of species of bacteria. In conditions of fermentation, sarcina ventriculi and yeasts may be present in large numbers ; in the healthy stomach they occur in much smaller numbers.

The intestine of the infant in whom feeding has become well established was found by Escherich to contain two principal species of bacteria—in the lower part of the intestine the bacillus coli communis, in the upper part the bacillus lactis aërogenes. More recently it has been shown that the stools of milk-fed infants, and to a less extent of adults, contain large numbers of anaërobic bacilli, which stain by Gram's method (bacillus bifidus—Tissier, bacillus acidophilus—Moro). These bacteria have not been fully studied.*

The number of bacteria in a milligram of human fecal matter has been estimated at from seventy thousand to thirty-three million. It is estimated that about one-third of the fecal matter of adults consists of bacteria. The small intestine of adults has been found by different observers to contain very different species.† The majority of these appear to have been introduced from the mouth in food or water. The bacillus

* Metchnikoff. Les Microbes Intestinaux. *Bulletin de l'Institut Pasteur.* May 15 and 30, 1903.

† Ford. Classification of Intestinal Bacteria, etc. Studies from the Royal Victoria, Montreal. March, 1903.

coli communis, however, occurs invariably in health not only in the intestine of man, but also in that of many animals, especially in the lower part.* The pyogenic micrococci very often occur in the intestine.

In the case of ruminant animals like the cow and sheep, the decomposition of cellulose, which forms so large a part of their food, appears to be affected by bacteria. Bacteria having this power are constantly found in the stomachs of ruminants. The best known species is that called bacillus amylobacter. It is questionable whether the products of the decomposition of cellulose have any nutritive value.

Pasteur some years ago expressed the opinion that if animals could be placed in such surroundings that bacteria could be excluded from the alimentary canal and the food, life would be impossible. This view has excited much controversy, and was apparently disproved by the experiments of Nuttall and Thierfelder. These investigators succeeded in removing guinea-pigs from the mother by Cæsarean section, and in keeping them alive in sterile surroundings, upon sterile food, so that the contents of the alimentary canal remained sterile. Schottelius, who worked with chickens, obtained contrary results, however; so that this interesting question is still undecided.

* Moore and Wright. Bacillus coli communis from Certain Species of Domesticated Animals. *American Medicine.* March, 1902.

CHAPTER V.

BACTERIA IN DISEASE.

To the physician and the student of medicine the study of bacteriology is interesting chiefly on account of the great importance attributed to bacteria in producing disease. The presence in an organism of one or a number of organisms of another species, which flourish as parasites upon the first, is a phenomenon of very wide occurrence in nature. It is, in fact, nearly universal. It may be observed among plants as well as animals, for example in the familiar galls seen on some of the higher plants, and mostly caused by the larvæ of insects harbored by the plant. We also find animals, such as tapeworms and the trichina spiralis, living as parasites upon other animals. The functions of the bacteria make them peculiarly suited to leading a parasitic existence. The fact that they possess no chlorophyll, and that they are therefore unable to form carbon compounds from the carbon dioxide of the atmosphere, renders it necessary for them to secure such compounds from preëxisting organic matter. Most of them, furthermore, flourish better when they are able to obtain nitrogenous food from organic matter rather than from inorganic salts containing nitrogen. Most bacteria find the necessary nutriment in the dead bodies of other animals and plants; they constitute what are known as saprophytes. But some of them flourish upon the living bodies of other plants and animals in whom they may produce disease.

The phenomena of disease, we shall find, are very largely due to the numerous waste products of the activities of bacteria, which act as poisons to the host.

The diseases of plants known to be caused by bacteria are not very numerous. Among them may be mentioned pear-blight, due to micrococcus amylovorus.* Among lower animals bacteria very frequently produce diseases—for example, chicken-cholera, symptomatic anthrax, erysipelas of swine, tuberculosis, anthrax and glanders.

Koch formulated certain rules which he considered must be complied with in order to prove that any microörganism was the cause of a particular disease:

First. That the organism should always be found microscopically in the bodies of animals having the disease; that it should be found in that disease and no other; that it should occur in such numbers and be distributed in such a manner as to explain the lesions of the disease.

Second. That the organism should be obtained from the diseased animal and propagated in pure culture outside of the body.

Third. That the inoculation of these germs in pure cultures, which had been freed by successive transplantations from the smallest particle of matter taken from the original animal, should produce the same disease in a susceptible animal.

Fourth. That the organism should be found in the lesions thus produced in the animal.

An *infectious disease* is a disease which is caused by a microorganism growing in the body of the animal having the disease. Such microörganisms are usually bacteria, but not always; for example, malaria is produced by a minute animal organism.

A *contagious disease* is one which is acquired from direct or indirect contact with an individual having the disease. Most contagious diseases are infectious, but infectious diseases are not necessarily contagious. The words are often used very loosely, and it is no longer possible or very desirable to draw the line

* See E. Smith. *Centralblatt für Bakteriologie*, etc. Zweite Abtheilung. Bd. V., p. 271; Bd. VII., p. 88.

sharply between them. *Fomites* are the materials on which the infectious material is conveyed.

A *miasmatic disease* is a variety of infection in which the microörganisms are not received from another case of the disease, but are supposed to have been derived from the external world, particularly through foul air. This word is less used than formerly.

The following is a list of the most important diseases of man caused by bacteria. The proof as required by the rules of Koch is not complete for all of them:

Tuberculosis,
Leprosy,
Glanders,
Anthrax,
Tetanus,
Malignant edema,
Bubonic plague,
Malta fever,
Erysipelas,
Suppuration and certain inflammatory conditions allied to it,
Epidemic cerebro-spinal meningitis,
Gonorrhea,
Chancroid or soft chancre,
Lobar pneumonia,
Influenza,
Diphtheria,
Typhoid fever,
Dysentery (not amebic dysentery),
Asiatic cholera,
Relapsing fever,
Rhinoscleroma (?),
Actinomycosis.

Malaria and amebic dysentery are caused by microscopic unicellular animal organisms (protozoa). It has been claimed that small-pox is caused by protozoa; this view has acquired added interest from the recent researches of Councilman. Recent work indicates that the "sleeping sickness" (of Africa) and some other diseases of tropical climates are caused by protozoa (see appendix).

Thrush and certain parasitic skin diseases are caused by fungi of more highly organized structure than bacteria.

In each of the following diseases there is good reason to think that the cause is some kind of microörganism, but it has not yet been discovered:

Syphilis, chicken-pox, measles, scarlet fever, German measles, mumps, whooping-cough, yellow fever, typhus fever, rabies, dengue.

Rheumatic fever and beri-beri would be placed in this list by many writers.

The causes of these diseases have been very carefully sought for by ordinary bacteriological methods with indecisive results. Some of them no doubt are due to bacteria. In recent years numerous observers have described a diplococcus or short streptococcus as the cause of rheumatic fever or acute rheumatism. In the case of yellow fever Sanarelli described an organism (bacillus icteroides) as its cause, but his view is not upheld by most of those who have worked on yellow fever.* The bacillus described by a number of observers as having been found in cases of whooping-cough may also be the cause of that disease.† Bacilli have also been described in cases of measles on several occasions. Lustgarten has described a bacillus found in the lesions of syphilis which resembles tubercle and smegma bacilli. More recently Joseph and Piorkowsky‡ have cultivated another bacillus from cases of syphilis; how much importance should be attached to it cannot as yet be stated. As Roux and Metchnikoff succeeded in 1903 in inoculating the chimpanzee with syphilis, it may be possible in the future to subject bacteria alleged to be the cause of syphilis to the test of animal inoculation. Recently Schaudinn and Hoffmann§ have found certain spirochætæ in syphilitic tissue, papillomata and the like, and it seems not improbable that the organism called by them Spirochæta pallida is the cause of syphilis. Metchnikoff and Roux found the same organism in the monkeys which they had successfully inoculated with syphilis. The organism is only seen by special methods of staining. It is likely that for some of the diseases mentioned other procedures than the usual methods of research will have to be devised in order that the

*Sanarelli. *La Semaine Médicale.* April 4, 1900. Reed and Carroll. *Journal Experimental Medicine.* Vol. V.

†See Czaplewski. *Centralblatt für Bakteriologie.* Bd. XXIV. 1898. P. 865.

‡*Centralblatt für Bakteriologie.* Vol. XXXI. 1902. Orig. p. 445. *Berliner klinische Wochenschrift.* 1902. Nos. 13 and 14.

§Schaudinn und Hoffmann. Vorläufiger Bericht über das Vorkommen von Spirochæten in syphilitischen Krankheitsprodukten und bei Papillomen. *Arbeiten aus dem kaiserlischen Gesundheitsampte.* Bd. XXII, Heft 2, p. 527. Berlin. 1905.

cause may be discovered. The protozoa may play a part in the etiology of some of them. Roux believes that contagious pleuropneumonia of cattle is due to a microbe so minute that it is barely visible with the highest powers of the microscope, so that its outlines and its morphology cannot be studied. The virus of this disease remains virulent after being passed through a Pasteur filter, showing that it is small enough to go through its pores. Similar experiments have succeeded with a number of other affections of animals (of which the best known is foot and mouth disease). The virus may pass through a Pasteur or Berkefeld filter of a certain coarseness, but is restrained by one sufficiently fine. The most important of the diseases in this class are rabies and yellow fever. Reed and Carroll found that the infective agent of yellow fever is in the blood, and that the serum could produce yellow fever in a non-immune person after filtration through a Berkefeld filter.* These facts suggest the possibility that failure to find the causes of some other diseases may lie in the fact that their organisms are so small as to be nearly or entirely invisible to the microscope.

Modes of Introduction.—There are various avenues by which bacteria may enter the body to produce disease. Infection of the embryo through the ovum or semen seems to be of rare occurrence. Syphilis (which may not be due to bacteria) is transmitted in this manner. The embryo may be infected through the placenta, although not commonly. The bacilli of typhoid fever and the pus-forming bacteria have been known to be conveyed through it. Tuberculosis may also be transmitted through the placenta; how frequently is still uncertain. Occasionally the exanthematous fevers are transmitted from the mother to the fetus.

*See Reed and Carroll. *American Medicine.* February 22, 1902. For an admirable review of this subject see Roux. Sur les Microbes dits Invisibles. *Bulletin de l'Institut Pasteur.* Vol. I., Nos. 1 and 2. Also Dorset. Invisible Microörganisms. United States Department of Agriculture, Bureau of Animal Industry, Circular No. 57. 1904.

The surfaces covered with thick, stratified epithelium are not likely to be penetrated by bacteria excepting by direct introduction through some wound or other lesion. This, for instance, is true of the skin, the mouth, the vagina and bladder. The infection of bubonic plague appears to be introduced most often by means of wounds in the skin. Bacteria more easily penetrate surfaces having a thin, columnar epithelium such as occurs in the intestines, the middle ear, bronchi and bronchial tubes, uterus and Fallopian tubes.

The thin, flat epithelial cells of the air-vesicles of the lungs, as would be expected, seem to be passed with comparative ease. On epithelial surfaces covered with cilia, as in the bronchi and bronchial tubes, the Eustachian tubes, the uterus and Fallopian tubes, the current toward the exterior created by the cilia acts beneficially in removing bacteria.

The tonsils and lymph-follicles of the intestines, especially the lymphoid tissue of the ileum and the vermiform appendix, are points where bacterial invasion frequently begins. The lymphoid tissue of the appendix may have some influence in predisposing to infection at that point and to appendicitis. On the other hand, it is certain that the progress of many infections is checked by the lymph-nodes. That is repeatedly seen in the ordinary post-mortem wound where the spread of the inflammation along the arm is checked suddenly at the elbow or axilla. The participation of the lymphoid structures in most infections is well known. How far this is a conservative process it is impossible to say.

In most cases of infectious disease a point of entrance for the bacteria may be discovered. As a rule, the invading microbes produce a lesion at the point where they are introduced, as in the familiar cases of boils and carbuncles when pyogenic bacteria enter the skin, or of the tubercles found in the lungs when the bacilli lodge in the respiratory tract. However, there are cases of septicemia and pyemia in which

the most careful search fails to reveal the place at which the bacteria entered. The bacilli of plague usually produce no reaction at the point of entrance.

It is probable that tubercle bacilli may pass through thin epithelial surfaces and lodge in the deeper structures underneath, where they produce definite lesions. For example, they may pass by the lungs and enter the bronchial glands, and form tubercles in that situation.

Experiments on animals have shown that bacteria may be very rapidly disseminated after their introduction. The inoculation of mice, for instance, with anthrax bacilli has been known to prove fatal, although the wound was washed immediately with the strongest antiseptic solutions or the part amputated within a few minutes.

The manner in which infectious agents reach human beings varies considerably. Generally speaking, the most important element will be found to be direct or indirect connection with another case of the same disease. W. H. Park was able to cultivate diphtheria bacilli from bedclothing soiled by the expectoration of diphtheria cases. Baldwin has shown that tubercle bacilli may be found on the hands of patients having pulmonary tuberculosis, especially those who expectorate on handkerchiefs. Winslow found bacillus coli communis on the hands of 5 per cent. to 19 per cent. of those examined; his observations suggest the possibility that typhoid bacilli can be carried in a similar manner.

Air.—Excepting under certain special conditions, the air will not contain the germs of disease. The dried pulverized sputum of cases of pulmonary tuberculosis may float in the atmosphere as dust which will contain tubercle bacilli. Flügge states that powerful expiratory efforts like coughing and sneezing may carry tubercle bacilli with small particles of secretion into the air, and that they may remain in suspension some time. The pus-producing bacteria may be present in dust. In-

fectious elements which happen to be present in the air will usually be attached to particles of dust. Wool-sorter's disease is a name sometimes applied to anthrax in man when acquired by those engaged in the work of handling wool, and in which the anthrax bacilli or spores may be conveyed to the lungs in dust.

The atmosphere in the vicinity of cases of the exanthematous fevers at times probably contains the germs of these diseases.

Water is the usual medium for the transmission of the infection in typhoid fever, and Asiatic cholera, and probably all forms of dysentery.

Milk from tuberculous cows may carry the bacilli of tuberculosis; it is of most importance in the case of young infants. Typhoid fever and cholera, and probably dysentery, scarlet fever and diphtheria are sometimes conveyed through the medium of milk. Bacteria may reach the intestines in *uncooked food*, fruit and vegetables.

The **Soil** is of importance in connection with tetanus and malignant edema, whose bacteria are frequently found in soil. Bacillus aërogenes capsulatus may occur in the soil, and may infect dirty wounds. The spores of anthrax bacilli are present in the soil of certain localities, and may produce anthrax in cattle.

Flies* and other insects and related animals are capable of carrying the bacteria of disease. Under suitable conditions flies play an important part in transporting the bacteria of cholera and typhoid fever from the excreta of these diseases to food substances, which they may contaminate. Flies which have access to tuberculous sputum may deposit tubercle bacilli on food.† To what extent diseases are disseminated by fleas, bedbugs and similar creatures is still uncertain.

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* Nuttall. Rôle of Insects, etc., in Disease. Johns Hopkins Hospital Reports. Vol. VIII. 1900.

† Lord. *Boston Medical and Surgical Journal.* Dec. 15, 1904.

In this connection it is proper to refer to certain diseases due to animal microörganisms. Malaria is conveyed from man to man by mosquitoes of the genus Anopheles,* and is probably transmitted exclusively in this manner. The parasite of malaria undergoes part of its cycle of development in man, and another part in the mosquito. Similarly, in Texas Fever, a disease of cattle, it has been shown by Theobald Smith that the parasite (a protozoön, Piroplasma) passes from cow to cow through the cattle-tick (Bophilus annulatus or bovis).† In surra, a disease chiefly affecting horses, and in the tsetse-fly disease of animals the parasite (a protozoön, Trypanosoma) is transmitted by the bites of flies.‡ It has recently been shown that the infectious agent of yellow fever may be introduced into man by mosquitoes of the genus Stegomyia. Under the administration of the United States army yellow fever was suppressed in Havana chiefly by measures intended to prevent the disease from being carried by mosquitoes. Equally good results have since been attained in controlling an epidemic of yellow fever at Laredo, Texas, in 1903,‖ and a great reduction in the mortality at Rio Janeiro, Brazil, has been effected.

Auto-infection.—It is possible for the bacteria of disease to be derived from the individual's own body—*auto-infection*. The microbes of lobar pneumonia, for instance, flourish in the mouths of a large number of people and under favoring circumstances may produce disease in the lungs or other parts. The bacillus coli communis, which constantly inhabits the intestines, may invade other organs and exhibit pathogenic properties when the way is opened up for it by other disease processes.

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* For detail concerning mosquitoes consult the book of Dr. L. O. Howard. McClure, Phillips & Co.

† See V. A. Moore. Infectious Diseases of Animals. 1902.

‡ Report on Surra. U. S. Bureau of Animal Industry. 1902.

§ Carroll. *Journal American Medical Association.* May 23, 1903.

‖ Guiteras. *Journal American Medical Association.* July 9, 1904.

Bodily Conditions that Dispose to Infection.—The development of an infectious disease may be favored by certain *bodily conditions*. Hunger, cold and exhaustion make the body more liable to the inroads of pathogenic bacteria; so also do anemia and chronic diseases. Those suffering from diabetes, as is well known, are especially liable to infection by the pus-producing bacteria and the bacillus tuberculosis. Dr. Roswell Park believes that prolonged anesthesia makes patients who have undergone operations more liable to surgical infections, and that absorption of bacterial poisons and auto-intoxication due to the products of disordered metabolism of the patient's own cells predispose to infection. Some of the above-mentioned conditions can be imitated in laboratory experiments. Hens in a normal condition are not susceptible to the anthrax bacillus, but Pasteur succeeded in making them contract anthrax by artificially cooling them. Frogs, on the other hand, which also are resistant to anthrax, may be made susceptible by keeping them at an abnormally high temperature. Rats were made more susceptible to anthrax by physical exhaustion produced by making them run a treadmill, and pigeons by starvation.

Abbott found "that the normal vital resistance of rabbits to infection by streptococcus pyogenes is markedly diminished through the influence of *alcohol*, when given daily to the stage of acute intoxication." It was less noticeable for bacillus coli communis, and not observed for staphylococcus pyogenes aureus. Pigeons and other animals have been made susceptible to anthrax by intoxicating doses of alcohol.

Climate and *altitude* appear to influence the liability to infection with the tubercle bacillus, which occurs less commonly in Colorado and some other elevated regions than in lower and more densely populated districts.

There are probably a great many other as yet obscure conditions affecting predisposition to infection.

Age.—In general, infants are more susceptible to infections

than adults, though apparently nearly exempt from the exanthematous fevers during the early weeks of life. Osteomyelitis is commoner in infants than in adults, as also is tuberculous meningitis.

How much influence is to be ascribed to *individual predisposition* in contracting or warding off infection is uncertain. Welch says: "The fact that some individuals are attacked, and others, apparently equally exposed to the danger of infection, escape, is not always due to any especial predisposition on the part of the former. It may be that the germs hit the one and miss the other, and we would have no more right to say that the former are especially predisposed than to say that those who fall in battle are predisposed to bullets and those who escape are bullet-proof." It is probable that the importance of an *hereditary tendency* to certain infections, notably tuberculosis, has been overrated.

Race.—The influence of racial predisposition is undeniable. For example, it is known that the negro race is much less susceptible to yellow fever than the white race.

Local conditions often have a most important influence in determining the occurrence of infections. In endocarditis the lesion usually occurs along the line of closure of the heart-valves, indicating that the point subjected to the greatest friction is the part of the endocardium most liable to infection. Regions where there is passive hyperemia are more vulnerable, as is seen in hypostatic pneumonia. Localities which have suffered from previous inflammation or irritation are rendered more liable to subsequent infection, as when the bladder or pelvis of the kidney containing a calculus becomes the seat of a suppurative cystitis or pyelitis.

Local conditions become of great importance in surgery. The surgeon can seldom be certain of dealing with a perfectly aseptic wound, and must rely to a large extent upon the power inherent in the fluids and tissues to prevent the development

of bacteria. It is important, therefore, to keep the resisting power of the tissues at the highest possible point. Injury of the tissues disposes the part to infection; so do strangulation and necrosis. In operating, it is to be remembered that hyperemic and edematous parts are more likely to become infected; so also are anemic regions. An infarct of the lung which was originally sterile may be infected with bacteria through inhalation, and undergo suppuration or gangrene. The presence of foreign bodies in the tissues disposes to infection. Injection of the staphylococcus pyogenes aureus into a rabbit's tissues is not always followed by suppuration, but if a foreign body, like a piece of sterilized potato, be inserted at the same time, infection is much more likely to occur. When lesions are produced in the internal viscera of animals by cauterization or crushing and bacteria then injected subcutaneously or into the blood, the bacteria lodge in the lesions and multiply.*

Amount of Infectious Material.—A large number of bacteria introduced into the body simultaneously will be more likely to produce infection than a small number. This factor is of less importance with organisms whose virulence is very constant than with those of more variable virulence.

Variability in the Virulence of Bacteria.—The occurrence of an infectious disease depends very largely upon the virulence of the bacteria. Any species of pathogenic bacteria may vary in virulence at different times. In some cases the virulence is not easily lost, as with the anthrax bacillus; in others the virulence is maintained in cultures only with difficulty, as in the case of the micrococcus lanceolatus (of pneumonia) and the streptococcus pyogenes. As a rule, the virulence is likely to be diminished in old cultures. It may sometimes be preserved better in the ice-chest than at the room temperature. The virulence of the anthrax bacillus becomes diminished if it

* Cheesman and Meltzer. *Journal Experimental Medicine.* Vol. III.

is cultivated at 42° to 43° C. Exposure to light and to oxygen tends to weaken the virulence; and also cultivation upon unfavorable media, such as those containing a small proportion of carbolic acid or certain other chemical germicides.

In laboratory work the virulence is usually maintained best by inoculating the bacteria from time to time into susceptible animals. Bacteria coming freshly from infected animals are likely to be highly virulent. The virulence may be increased by beginning with an especially sensitive animal like a very young guinea-pig, and progressively inoculating into less sensitive animals. The infection of relatively insusceptible animals may sometimes be produced by the injection of a very large dose of the bacteria. The addition of the toxic products of the bacteria, which may be obtained by using large doses of cultures in bouillon, makes infection more likely. Cultivation on a particular medium may maintain or increase the virulence.

Finally, the combination of two or more kinds of bacteria may produce infection when neither one would do so alone. On the other hand, it is said that the fatal effects of an inoculation of virulent anthrax bacilli into a susceptible animal may be averted if the animal be inoculated with a culture of bacillus pyocyaneus shortly afterward.

Mixed Infection.—It is not uncommon in disease to find two kinds of bacteria associated together, producing a mixed infection. In diphtheria, very frequently, the bacillus of diphtheria is found to be accompanied in the membrane by the streptococcus pyogenes. The course of the diphtheria may be modified in this manner. The term *secondary infection* is rather loosely used. It is sometimes employed to designate an infection occurring in an individual, the resisting power of whose tissues has been weakened by some chronic organic disease. Such an infection is often called a *terminal infection.* Terminal infections are very common in cases of car-

cinoma, chronic nephritis, arteriosclerosis, and in many other diseases.

Concerning terminal infections Osler says: "It may seem paradoxical, but there is truth in the statement that persons rarely die of the disease with which they suffer. Secondary infections, or, as we are apt to call them in hospital work, terminal infections, carry off many of the incurable cases in the wards."

The term *secondary infection* is also used for the modification of an infectious process which has been in existence for some time, by infection with a second variety of bacteria. That takes place, for instance, in pulmonary tuberculosis, when the invasion of the already tuberculous lungs by the pyogenic micrococci assist in the formation of cavities. In this sense it will be seen that the term secondary infection is used as a name for a variety of mixed infection. In the secondary, mixed and terminal infections, the bacteria which enter secondarily are likely to be of the pus-producing varieties, especially the streptococcus pyogenes.

As to the mechanism which bacteria make use of in order to produce disease, according to our present knowledge, they work chiefly through the poisonous substances formed by them and deposited in the bodies of the persons suffering from the disease. The theory that bacteria have an important influence through the destruction of substances taken by them from the body of the patient for food is no longer entitled to much weight; neither are we able in most cases to account for the phenomena of disease by any mechanical action on the part of the bodies of bacteria. That such action does occasionally take place may be seen in experimental anthrax in mice, where the blood-capillaries of the liver and kidneys may be completely plugged with masses of anthrax bacilli. The diseases in which the circulating blood is swarming with bacteria are much commoner in the lower animals than in man.

Toxemia.—By toxemia is meant the absorption of poisonous

bacterial products from a localized point of invasion, and their dissemination throughout the body by means of the circulation. We see typical toxemias in diphtheria and tetanus. In surgery the term *sapremia* is used to cover a similar condition of affairs when the absorption proceeds from a wound or denuded surface, as may happen in the puerperal uterus.

Septicemia.—In septicemia there is not only absorption of bacterial poisons, but the bacteria have invaded the living tissues and the blood (though not producing metastatic abscesses). Bacteriologists usually employ the word septicemia to describe the wide dissemination of bacteria through the body and the presence of a large number of them in the circulating blood. In this sense septicemias are less common in man than in such diseases as anthrax in the lower animals. Typical septicemias in man are found in relapsing fever and certain cases of bubonic plague. For pyemia, see the article on Suppuration, Part IV.

The principal agencies in effecting *recovery* from infectious diseases are the destruction of the bacteria by the cells of the body (phagocytosis), the development of new substances which neutralize their action (antitoxins) and the presence or formation in the body of substances which destroy bacteria (lysins). These phenomena are discussed in the chapter on Immunity. A factor of less importance is the elimination of bacteria by the excretory organs. Investigators who have made experiments on animals disagree as to whether or not the bacteria which have been injected into the body appear in the urine before they have damaged the structure of the kidney. In typhoid fever, the bacilli of typhoid may occur in the urine in great numbers; the condition of the kidney in the generality of such cases has not thus far been determined. The extent to which the excretory organs act in eliminating bacterial toxins is not yet known. Some bacteria, as has already been stated, may, in the end, produce substances that are inimical to their own growth.

CHAPTER VI.

BACTERIAL POISONS.*

It is now generally accepted that in most, if not all of the infectious diseases, the principal symptoms and lesions are to be attributed to the action of poisonous substances formed by the bacteria. Even in those cases where poisonous substances are not demonstrable in cultures to any great extent, there is reason to believe that the bacteria in such cases may, nevertheless, produce poisons in the animal body. According to present views the part that bacteria play can be understood best by recalling the work of the saprophytes in producing fermentation and putrefaction. It has already been shown that the poisoning that comes from eating decomposed meat, fish or cheese usually results from poisons which bacteria have elaborated in the course of their growth. In infectious diseases the bacteria grow inside of the body and form their poisons *in it;* not before their introduction into it, as in these cases of poisoning with spoiled food. If it were possible for the cells of ordinary yeast to grow in the living human body and to produce alcohol from the grape-sugar of the body-fluids, the person so infected might be expected to suffer from alcoholic intoxication as long as the infection lasted. This illustration although not entirely accurate may help to make clear what does happen in an infectious disease due to bacteria, where poisons formed in a manner analogous to the formation of alcohol produce intoxications analogous to alcoholic intoxication. Certain infectious diseases afford examples of poisoning by

* For a full consideration of this subject see Vaughan and Novy. The Cellular Toxins. 1902.

bacterial products in an extremely marked manner. In tetanus the local wound may be trifling, and in itself utterly incapable of giving rise to the violent muscular spasms from which the patient suffers in consequence of the powerful poison which the tetanus bacillus forms at the point of infection. In diphtheria, although the condition in the throat may be one of severe inflammation, it is of itself insufficient to explain the profound prostration and other symptoms of general poisoning which the case manifests.

Bacterial poisons are diffused through the culture-medium or they may be retained in the bodies of the bacteria. Consequently, they are classed as extracellular and intracellular. In cultures of the diphtheria and of the tetanus bacilli the culture-medium contains the poison, and injections of the broth in which these organisms have been grown produces these diseases just as promptly and effectually as injections of the bacteria themselves. Even when the bacteria is these cultures are entirely removed by filtration through porcelain filters, the filtrate reproduces the diseases with all their symptoms just as characteristically as the unfiltered cultures. On the other hand, in certain bacteria the poisons are contained in the bodies of the bacteria, and are not liberated into the culture-medium. They are only set free by breaking up the cells, either mechanically, by grinding in a mortar, or by disintegration in some other way. The disintegration of these bacteria in the animal body is probably the way in which certain of them cause disease. Typhoid bacilli and cholera spirilla probably act in this way.

The first bacterial poisons to be studied thoroughly were those called ptomaines. Observing the poisonous effects which follow the injection into animals of certain ptomaines derived from bacterial cultures, it was suggested that similar ptomaines, formed by the action of bacteria in the living body, might account for the symptoms of many of the infectious diseases. The ptomaines were most readily studied because

of the comparative facility with which they could be isolated in a condition of purity, where their exact chemical nature could be determined. They were found to be basic compounds derived from nitrogenous material.

A group of substances of a similar nature called leucomaines has been discovered, which are formed within the body by faulty metabolism, and not by the action of the bacteria.

Further study has demonstrated, however, that the characteristic features of the infectious diseases are not due to ptomaines, but to certain poisonous bodies to which the name toxin has been applied. Toxins have not been obtained yet in a pure state, and consequentially their exact chemical character has not been determined; but much has been learned in regard to their physiological action, and more information in this direction is constantly being obtained by experiments. They have very marked characteristics and they do not act like ordinary poisons, but behave as if they had the power of reproduction. An ordinary poison, such as arsenic, strychnia and the like, begins to act as soon as it is absorbed—there is no period of incubation. The toxins, on the contrary, have a distinct period of incubation. If an animal is given a fatal dose of arsenic or strychnia, it succumbs within a comparatively short time; it is at most a matter of a few hours. But if an animal is injected with a fatal dose of the toxin of tetanus it takes some time, often several days, before any symptoms develop, and moreover the animal may remain alive for days afterward. In some respects the toxins resemble the physiological ferments, ptyaline, pepsin and the like; but they differ from these in that the physiological ferments are not themselves used up in the process of fermentation, whereas the toxins are used up in the production of disease. After starch has been converted into sugar by ptyaline the ptyaline may be recovered and used over and over again to convert more starch; but after tetanus toxin has produced tetanus in an animal it has become

firmly united to the cells. Toxins, therefore, are very peculiar bodies, behaving like ferments in requiring considerable time to produce effects, and acting like unorganized poisons in being used up in the tissue changes which they produce. Certain substances derived from the vegetable kingdom behave in the same manner as bacterial toxins; ricin, abrin and robin are examples of these. The poisons of scorpions and snakes are also poisons which act like toxins. Other properties of toxins will be considered in connection with antitoxin.

Although, as has been stated, the toxins have not been isolated in a pure condition, they have, nevertheless, been obtained in some cases in an extremely concentrated form. Brieger and Cohn obtained a toxin from tetanus bacilli of which 0.00000005 gram killed a mouse weighing 15 grams. Roux and Yersin obtained a toxin from diphtheria bacilli of which 0.00005 gram was capable of killing a guinea-pig. These figures indicate the extremely poisonous character of these toxins. Such properties permit bacteria growing in a comparatively limited area to act upon parts of the body remote from the focus of infection.

A curious and unexplained effect of some toxins is the production of minute areas of necrosis in certain viscera, as the liver. Such "focal necroses" have been observed to be formed by the poisons of the bacilli of diphtheria, of typhoid fever, and of the Micrococcus lanceolatus of pneumonia, and following the injection of abrin and ricin.

Besides the poisonous substances produced by the bacilli of diphtheria and of tetanus, toxic substances have been obtained from the spirillum of cholera, the bacillus of typhoid fever, the Bacillus coli communis, the bacillus of bubonic plague, Bacillus pyocyaneus, Streptococcus pyogenes, and Staphylococcus pyogenes aureus. The extract from cultures of tubercle bacilli, called tuberculin, and that from glanders bacilli, called mallein, will be spoken of in connection with the bacteria themselves. Vaughan * has succeeded in cultivating anthrax bacilli, colon bacilli, and other bacteria on large surfaces of solid media, so as to secure quantities of the bacterial cells sufficient for extensive chemical tests. The toxin of the colon bacillus proved to be a very stable substance, and resistant

* *Journal American Medical Association.* September 3, 1904.

to heat. Most toxins become inactive at comparatively low temperatures (60° to 70° C.).

Other physiological properties of the toxins will be brought out in connection with the discussion of immunity.

There is good reason on both clinical and experimental grounds to believe that toxic substances are formed by the Micrococcus lanceolatus of pneumonia.

In connection with bacterial poisons another class of bodies may be conveniently described; these are *agglutinins*, *lysins*, and *precipitins*. These bodies will be referred to again in connection with antitoxin in the next chapter.

Agglutinins.—The blood-serum of human beings as well as of animals suffering from certain diseases has the power of causing the bacteria of the disease from which the individual has recovered to clump into larger or smaller masses in liquid cultures to which the serum is added. The same phenomenon is observed in the serum of animals injected with repeated doses of cultures. This is due to certain substances called agglutinins. The reaction, while it is more or less specific, is not as strictly so as was formerly thought, for it has been found that a given agglutinin may cause clumping of a group of nearly related bacteria; such an agglutinin is called a group agglutinin; and, moreover, under certain circumstances the bacteria fail to clump in the blood of patients suffering from a given disease. Again, in some cases the serum in a certain disease will clump bacteria that are not concerned in the production of the disease. Even normal serum will sometimes clump bacteria. The serum from a given disease is said to be homologous with the bacteria causing the disease and heterologous from other bacteria, and the bacteria are said to clump or not to clump with homologous sera, as the case may be. Bacteria are also homologous or heterologous in the same sense toward sera.

Park* has recently found in this connection that homologous

* W. H. Park. Proceedings of the New York Pathological Society. Vol. IV., Nos. 1 and 2. February and March, 1905.

sera lose their property of agglutination, but recover this when cultivated upon the ordinary culture-media. Weil* obtained a culture of typhoid bacillus from an abscess in the thyroid of a typhoid convalescent which did not agglutinate with the patient's serum nor with other homologous sera.

Agglutination has been found to take place even spontaneously in cultures. Still, it is, after all, more or less a specific reaction, and is employed as an aid in the diagnosis of typhoid fever, where it is spoken of as the Widal or Gruber-Widal test. Under proper precaution it is valuable in this special case, and will be referred to again in connection with the description of the typhoid bacillus.

Other bacteria which agglutinate with the homologous sera are: Spirillum of cholera, B. pyocyaneus, B. proteus, B. coli communis, Micrococcus melitensis, B. mallei, B. tuberculosis, Diplococcus pneumoniæ, B. pestis bubonicæ, and B. dysenteriæ. Trypanosomes also agglutinate with homologous sera.

Lysins.—There are certain substances found normally present or produced artificially in the blood which have the property of breaking up foreign red blood-cells introduced into the circulation or into the blood-serum outside the body. This is not only true of red blood-cells, but certain bacteria also become broken up when introduced into the blood of certain animals. This process is spoken of as cytolysis, and when occurring in red blood-cells, is called hemolysis; in bacteria, bacteriolysis. The substances causing cytolysis are called lysins. As already stated, lysins for certain foreign cells are normally present in the serum of certain animals; thus, human red blood-cells are disintegrated by sheep's serum, rabbit's blood-serum disintegrates anthrax bacilli, and numerous other examples exist of lysin normally present in blood-serum. But whether normally present or not, specific lysins, like specific agglutinins, are made to appear in the blood-

* Weil. *Ibid.*

serum of animals by injecting these with several doses of suspensions of cells. Hemolysin results from injecting an animal with the red blood-cells from another animal, bacteriolysin from the injection of bacteria. Hemolysins and bacteriolysins are quite sharply specific. A rabbit injected with a suspension of red corpuscles from the blood of a guinea-pig furnishes hemolysin which destroys guinea-pig's red cells. A guinea-pig injected into the peritoneal cavity with repeated small doses of the cholera spirillum furnishes a peritoneal fluid containing a bacteriolysin specific for the cholera spirillum. Still, group lysins, like group agglutinins, are also found, for while lysis takes place more promptly and in smaller amounts with the cells of the same species of animal or with the same kind of bacteria with which the animal furnishing the cytolytic serum has been injected, it also occurs in a less marked degree with cells from nearly related animals or with nearly similar bacteria.

Precipitins.—Precipitins are bodies which develop in the serum of animals which have been given subcutaneous injections of albuminous substances, and which, added to solutions of the albumen with which the animals have been injected, cause these to become cloudy and finally form a precipitate. In other words, the serum of an animal injected with a serum or other kinds of albumen causes precipitation in the homologous form of albumen.

Thus, a rabbit may be immunized or adapted to hen's egg-albumen. The rabbit's serum will then precipitate the hen's egg-albumen. It may, however, imperfectly precipitate albumen from the egg of a species closely allied to the hen.

Again, a rabbit injected a few times at intervals of a day or two with human blood-serum furnishes serum which even in small quantities causes precipitation even in a weak solution of human blood-serum, such as may be obtained from old dried blood-spots.

The reaction is very sensitive, the immune serum, as the serum from the injected animal is called, causing clouding in solutions containing very small traces of the homologous serum. Rabbits are usually employed for the production of immune sera for the precipitation reaction, and seem specially adapted to the purpose.

The reaction is of great value in determining the kind of blood in any doubtful case, and is applied practically in forensic medicine to determine the character of suspicious blood-stains.

In the precipitin reaction, as this is called, there is group precipitation, it is true. Thus, human and monkey's sera react with the same precipitin and dog's and wolf's sera respond to the same precipitin. Bacterial precipitins have also been obtained by injecting animals with bacteria. In this case filtrates obtained by filtering bacterial cultures or suspensions through porcelain filters give a cloud, with ultimate precipitation, when treated with a drop of homologous serum.*

* Norris. The Bacterial Precipitins. *Journal of Infectious Diseases.* Vol. I., p. 463. 1904.

CHAPTER VII.

IMMUNITY.

Studies in immunity have led to remarkably uniform results in so far as the facts are concerned. All agree on the actual observations, both of the processes which take place spontaneously in nature, as well as of those which follow in intentional experiments. There is, however, great difference of opinion upon the interpretation of these phenomena, and several opposing theories have been advanced in regard to the mechanism concerned, each theory finding very eminent supporters.

In view of these facts, it is necessary, in discussing immunity, to give a definition covering its broadened application, to cite the observations which have been recorded and to present the prevalent explanations offered by the various authorities, omitting various theories now abandoned.

Immunity as formerly studied embraced only considerations of the insusceptibility of individuals or of races to an attack of a given infectious disease. But the modern conception is broader than this, and it is no longer confined to immunity proper, but extends to certain other processes which have been found to bear a close resemblance, in certain respects, to immunity, and to be governed by laws very similar to those which govern the latter.

Immunity at present is made to include besides insusceptibility to infection—*i. e.*, resistance to the invasion of living bacteria—the processes concerned in the forming of the antibodies, antitoxins proper, antiagglutinins, antilysins and antiprecipitins.

Resistance to Infection.—Immunity from infectious diseases is either natural or acquired, active or passive.

By natural immunity is meant the inherited power possessed by certain races or individuals, independently of size, habits or surroundings, to resist infection to which other races or individuals are subject. This is illustrated by many examples. Rats are ordinarily insusceptible to anthrax, whereas mice, guinea-pigs, sheep, cattle—in short, most animals—are very susceptible. Mice are not susceptible to diphtheria poison on inoculation, while horses, sheep, goats, guinea-pigs and many other animals are very susceptible. Even very nearly related species or varieties often show difference in susceptibility. House-mice are susceptible to mouse septicemia, field-mice are not. With glanders the reverse is the case in these species of animals. Negroes are insusceptible to certain diseases to which white persons are very subject, and also the reverse.

Instances of individual immunity are seen in every epidemic, where persons escape when they are under the same conditions as those who have contracted the disease. Instances also occur in which nurses and others thrown with cases of highly infectious diseases escape. Some of these cases, it is true, belong more properly to the category of immunity acquired by recovery from an attack, since nurses and others thrown in contact with an infectious case may suffer a very mild attack or even, it is probable, become immunized without showing any symptoms of disease. Nurses and physicians have been found with diphtheria bacilli in their throats and yet not showing any symptoms. Of course, these persons may have had natural immunity, but it is equally possible that they may have become gradually immunized.

Acquired immunity follows recovery from a spontaneous attack of certain diseases, and it also results from intentional inoculation. Immunity after recovery is so familiar that no

illustration is necessary. But not every infectious disease leaves immunity behind; many, on the contrary, are followed by no increased resistance, or if there is increased resistance, it is very transitory; some even tend to increase susceptibility. Examples of infectious diseases leaving no lasting immunity, and even in some cases apparent increased susceptibility, are erysipelas, diphtheria, influenza, pneumonia, gonorrhea. In those cases in which immunity follows recovery from a spontaneous attack of an infectious disease the process is probably the same as that which takes place after intentional inoculation.

Immunity acquired after intentional inoculation is produced either by inoculation with material which produces a mild type of disease, as in vaccination for small-pox; or by giving at short intervals inoculations of the disease-producing virus of graded strength, beginning with greatly attenuated material, and followed with stronger and stronger material, as in inoculation for hydrophobia and anthrax; or by injections of larger and larger amounts of bacterial poison, as in the production of antitoxin for diphtheria and tetanus; or, finally, by the injection of the antitoxin formed in the blood by the method last mentioned, as in the treatment of diphtheria and tetanus, for this, after all, is, in a way, a process of immunization.

Small-pox and Vaccination.—The origin of vaccination against small-pox with the virus of cow-pox has been described in the historical sketch (p. 10). The nature of the protection furnished by this virus has been the subject of much controversy. The opinion of the present day inclines to regarding vaccinia as small-pox which has been modified by passage through a relatively insusceptible animal. Certainly there are many analogies between the protection against small-pox afforded by vaccination and the other examples of artificial immunity mentioned below.

This question cannot be settled with certainty until the

organisms causing small-pox and vaccinia have been isolated in pure culture. Their identity and mode of action may then be determined.

Small-pox has been inoculated into calves and passed through other calves in succession, producing finally an eruption indistinguishable from cow-pox. Lymph taken from such calves has been used successfully to vaccinate children. Not only does cow-pox protect against small-pox, but it has been shown that small-pox protects against cow-pox.

Immunity Produced by Inoculation with Bacteria of Diminished Virulence.—Pasteur conceived the idea of attenuating the virulence of the bacilli of fowl-cholera by prolonged exposure to the air. He made use of the attenuated virus as a vaccine against the disease.

A nearly similar principle was shortly afterward applied by him to the preparation of a vaccine against anthrax. When anthrax bacilli were cultivated at a temperature of 43° C., Pasteur obtained bacilli of very slight virulence. Such bacilli did not produce death when inoculated into animals that were ordinarily susceptible. Yet animals that were vaccinated with this virus were able afterward to resist inoculation with fully virulent anthrax bacilli. (See Bacillus anthracis, Part IV.)

In the case of erysipelas of swine (French, *rouget;* German, *Schweinerothlauf*) Pasteur secured bacilli of diminished virulence by injecting virulent bacilli into relatively insusceptible animals. The animal used was the rabbit. The bacilli were passed through several rabbits in succession. Cultures taken from the last of the series produced a milder form of the disease and an amount of immunity the value of which is in dispute.

In still another disease, black-leg of cattle or symptomatic anthrax (French, *charbon symptomatique;* German, *Rauschbrand*), an attenuated virus is secured by the use of heat. The pulp from the infected muscle of a diseased animal,

containing the bacilli, is squeezed from it and heated to a temperature of 95° to 99° C. for six hours. The dried material mixed with water constitutes the vaccine. The Department of Agriculture of the United States now furnishes this vaccine free to farmers. The results of this method are said to be very gratifying.* In the human disease, bubonic plague, a nearly similar procedure has been proposed by Haffkine. To protect against plague, cultures of plague bacilli are used which have been previously sterilized by heat, with carbolic acid added as a preservative. (See section on Bubonic Plague, Part IV.)

Inoculation Against Rabies or Hydrophobia.†—The immunity produced in this case probably depends upon principles similar to those underlying the examples related on the preceding pages. But this question cannot be regarded as settled until the organism of rabies has been isolated and cultivated. Attempts to discover this organism have, as yet, been futile, though certain minute bodies, bodies of Negri, have been observed within ganglion-cells of the central nervous system from cases of rabies, and it has been claimed that they are protozoa and the cause of the disease. Whether this is true or not, Negri bodies make a most valuable means of rapid diagnosis.

Pasteur discovered that rabies could be produced in animals by inoculation under the dura mater with portions of the spinal cord of a dog suffering from hydrophobia. He also found that successive passages through a series of rabbits greatly increase the virulence of the virus, as indicated by a much shorter period of incubation after inoculation. The first rabbit of the series inoculated with the "street" rabies

* See Recent Annual Reports, Bureau of Animal Industry, U. S. Department of Agriculture.

† For a review of recent works on rabies see Remlinger. *Bulletin de l'Institut Pasteur.* II., Nos. 19 and 20. 1904.

virus—*i. e.*, from a spontaneous case in a dog—dies in about two weeks, and each succeeding rabbit dies in a shorter and shorter time until ultimately the incubation period is reduced to six or seven days. Beyond this the strength of the virus cannot be increased, and is called virus "*fixe*," or the fixed virus. Pasteur found, moreover, that the cord of the rabbit which has attained this degree of virulence is attenuated by various agencies, notably by drying, and that animals injected with this attenuated virus can withstand inoculations of more potent virus. By drying for various lengths of time a series of "vaccines" of exactly graded potency is obtained, and starting with the vaccine of least potency an animal can be inoculated with increasingly potent virus until it will withstand inoculations of the *virus fixe* itself.

Omitting all but the chief details, the vaccines against hydrophobia are prepared as follows:

The cord of a rabbit dead from the subdural inoculation of *virus fixe* is hung up in a long glass cylinder in the bottom of which is placed potassium hydrate. The cylinder is placed in a cool place, and every day small bits of the cord are cut off and preserved in a vial of glycerin. The virus which has been dried for thirteen or fourteen days is no longer capable of producing hydrophobia in rabbits, but an animal inoculated with it can withstand inoculation with the cord dried for a shorter time, and after inoculation with the latter withstands inoculations with cord dried for a still shorter time.

In human beings it is customary to start with the virus which has been dried for nine or ten days, injecting subcutaneously emulsions of the dried cord, and, if time permits, to give an inoculation every day with virus dried for a shorter and shorter time. As the incubation period for human beings bitten by a mad dog is quite long,—about six or eight weeks,—there is ample time to run in all the inoculations if these are begun promptly, and if in this way the individual is made to with-

stand the *virus fixe*, it is more than probable that the weaker virus from the dog will not be able to cause any disease.

Where much time has elapsed after the bite of the mad dog, it is sometimes the practice to give three or more injections of increasing strength every day.

These inoculations against hydrophobia have proved to be most valuable, as the large number of reports from various Pasteur institutes in various parts of the world abundantly prove. According to statistics, collected by Ravenel, based on many thousands of cases, the mortality from rabies in those so treated is less than one per cent.*

In all cases where a human being has been bitten by a dog that is suspected of having hydrophobia the individual should submit himself to the Pasteur treatment as soon as possible, if it is feasible to do so. In order to find out whether the dog has hydrophobia, the animal should be killed and the medulla or the head sent to some one competent to make the examination. The examination consists in looking for the Negri bodies and in subdural inoculation of rabbits. If the cord of the dog can be obtained, the intervertebral ganglia will show round-cell infiltration. It is safer not to wait for the examination if the dog is probably mad, though there is abundant time usually even to wait for the results of the inoculation of rabbits; still this is not to be recommended.†

Great care must be taken that the operator may not accidentally infect himself.

Antitoxins.—The first efforts to point out the way along which antitoxins may be produced were made by Salmon and Smith in 1886. In their experiments pigeons were injected with filtrates from cultures containing the products resulting

* See Ravenel and McCarthy. *University of Pennsylvania Medical Bulletin.* June, 1901. Also editorial in *Philadelphia Medical Journal.* March 14, 1903. And Mohler. Twelfth Annual Report of the United States Bureau of Animal Industry.

† Varanus A. Moore. Infectious Diseases of Animals.

from growth of the hog-cholera bacillus. Such pigeons were found to be immune to this bacillus, which is pathogenic for ordinary pigeons.

As was stated in the last chapter, bacterial poisons may be of two sorts. In one group the poisons occur chiefly within the bodies of the bacteria. This group seems to contain the majority of the pathogenic bacteria. Methods of protection against infections caused by them will be considered hereafter. But anti-endotoxins, or substances which counteract intracellular or endotoxins, have not yet been satisfactorily produced.*

In the other group the poisons do not, for the most part, remain in the bodies of the bacteria, but are readily diffused from them into their surroundings. It is for the bacteria of the latter group that antitoxins have been obtained. Its most important members are the bacilli of diphtheria and tetanus. Their poisons may be found in the culture-media in which they have grown. The principle employed in preparing antitoxins was established by Behring, who found that by injecting susceptible animals with increasing amounts of toxin he produced in the blood-serum of the injected animal certain changes which made the serum capable of counteracting the toxin when injected into other animals. Thus, a sheep treated with increasing doses of diphtheria toxin, beginning with very small amounts, furnishes blood-serum which protects other sheep or guinea-pigs or other susceptible animals from fatal doses of diphtheria toxin. In practice the bacilli are cultivated in bouillon. The cultures are freed from all living bacilli by filtration. The liquid filtrate contains the toxin. This filtrate is injected into healthy susceptible animals, in increasing doses. Usually the horse is used, since large quantities of blood can be drawn from this animal on

*Kolle and Wassermann. Handbuch der pathogenen Mikroorganismen. Bd. I., p. 373. Jena, 1903.

account of its size, and, moreover, the horse is very susceptible. Insusceptible animals cannot be made to yield antitoxin, at least of any appreciable strength. Eventually enormous doses of toxin are given, and the animal acquires a high degree of immunity. The blood of the animal is withdrawn, taking care to avoid contamination, and the serum allowed to separate in the refrigerator. The serum of the blood is drawn off and constitutes the antitoxin. The use of antitoxin has been eminently successful and revolutionized the treatment of diphtheria; and it has given partial success in tetanus with an antitoxin prepared by injecting horses with increasing amounts of tetanus toxin. (See the description of the bacteria of these diseases.)

Ehrlich discovered that the vegetable toxins, abrin and ricin, behave in a manner very similar to soluble bacterial poisons when injected into animals, and that by their injection an immunity for the same poisons may be secured. Ehrlich also found that the milk of animals which had been immunized with increasing doses of abrin and ricin confers immunity upon sucklings. In most cases we look to the blood-serum for the immunizing agent.

There is little, if any, analogy between the tolerance acquired in this manner from bacterial and other toxins and that which victims of the morphine and cocaine habits have for immense doses of these drugs, for no bodies resembling antitoxins are obtained from animals that have been accustomed to such drugs.

Active and Passive Immunity.—The kind of immunity which results from the injection of substances from immunized animals is called "passive immunity." Diphtheria and tetanus antitoxins produce passive immunity. "Active immunity" may be brought about in several ways:

(1) By a spontaneous attack of an infectious disease; (2) by an attack excited artificially through inoculation with small doses of virulent cultures, or (3) by the administration of large doses of attenuated cultures; (4) or by the injection of

bacterial products (toxins) freed from the bacteria themselves. Pasteur's methods of protective inoculation for anthrax and other diseases, and Haffkine's injections for bubonic plague, produce active immunity. Active immunity is usually more enduring than passive immunity. But passive immunity, resulting, as it does, from the direct introduction of antitoxin, is brought about more quickly than active immunity.

Theories of Immunity.

Phagocytosis.*—Metchnikoff described under the name "phagocytosis" immunity and recovery from bacterial invasion. This theory is based on the well-known fact that certain cells of the body have the power of surrounding and ingesting foreign substances. The cells in question are chiefly polynuclear leukocytes, but to some extent other leukocytes and endothelial and other cells are also concerned. The polynuclear leukocytes are the cells which destroy bacteria, and Metchnikoff now calls these *microphages;* other phagocytes he calls *macrophages.* There are many examples of phagocytosis which have been observed. The phagocytes of the lungs constantly take up small bits of carbon inhaled with the air. Particles of carmine injected into the tissues will later be found within phagocytes. After a hemorrhage, phagocytic cells may be found containing red blood-corpuscles or particles of blood pigment. The presumption is that phagocytic cells serve to remove irritating and foreign bodies and to destroy them. Metchnikoff showed that phagocytes also absorb bits of degenerating or useless tissue. Such particles disintegrate, and they are digested and become a part of the protoplasm of the phagocytes. This process is seen when the tail of the tadpole shortens. The superfluous part is absorbed, at least in part, by phagocytic leukocytes. Metchnikoff's earlier observations were made largely on the invertebrates, whose transparent bodies may

* Greek, φαγειν, to eat; κύτος, a cell.

be studied while living. One illustration was furnished by a small crustacean (*Daphnia* or water-flea), which was often infected with a fungus. Some infected individuals died, others recovered. Metchnikoff found that the cells of the fungus might be ingested and destroyed by the leukocytes of the *Daphnia*. He described the history of this disease as a contest between the parasitic cells and the phagocytes, in which either might succeed. Similarly, when anthrax bacilli were introduced into frogs, which are immune from anthrax, the bacilli were ingested by the frog's leukocytes. Metchnikoff* contends that this function of leukocytes and other phagocytic cells constitutes the principal defence of the body against bacteria.

Other investigators also have seen bacteria enclosed within the bodies of leukocytes. It has been urged by some that the bacteria are already dead when the leukocytes devour them, but Metchnikoff showed that these enclosed bacteria are still alive, for they produce disease when introduced into fresh animals; so they are apparently not injured before they are taken up. In other cases, as with the gonococcus, which is commonly found enclosed within leukocytes, it is quite evident from their appearance that the bacteria retain their full vigor after being ingested.

It is well known that a suppurating part contains large numbers of leukocytes, and one of the most characteristic events in the inflammatory process is the migration of leukocytes to the point of irritation. This indicates a positive chemotaxis for leukocytes on the part of substances in the inflamed area. Metchnikoff believes that the function of these leukocytes is to destroy the bacteria and to arrest their further progress. On this theory bacteria have often been likened to an

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* Metchnikoff. Comparative Pathology of Inflammation. Trans., Starling. 1893.

invading army and the leukocytes or phagocytes to a force designed to repel their attacks.

It is certain that in some infectious diseases the number of leukocytes, chiefly of the polynuclear neutrophilic variety, in the circulating blood is increased (leukocytosis). This is the case usually in lobar pneumonia and acute suppurative infections. In other infectious diseases there is no leukocytosis; for example, tuberculosis, typhoid fever and malaria. It is interesting to observe that in trichinosis, and more rarely in infection with other animal parasites, the eosinophilic leukocytes become much more numerous in the blood than normally.

Experiments have been made by various investigators, consisting in the production of local leukocytosis, and studying the effects of the leukocytes thus brought together upon bacteria. The injection into the pleural or peritoneal cavity of various substances, notably nucleic acid, or aleuronat suspen sions, calls forth a great accumulation of leukocytes, and these masses of leukocytes have been used for the purpose of observing the phenomena of phagocytosis both inside and outside the body.*

In operations upon the abdominal cavity the production of artificial leukocytosis in the peritoneal cavity previous to operations has been suggested and tried with apparent success on the ground that if any bacteria entered during the operation they would be destroyed by the phagocytes.†

Wright and Douglas‡ found that certain substances prepare

* Gustav F. Ruediger. The Mechanism of Streptococcus Infection. *Journal of the American Medical Association.* No. 3. January 21, 1905. P. 198. Ludvig Hektoen and Gustav F. Ruediger. Studies in Phagocytosis. *Journal of Infectious Diseases.* Vol. II., No. 1, p. 128. January, 1905.

† Von Mikulicz. Versuche über Resistenzvermehrung bei Magen- und Darmperforationen. *Archiv für klinische Chirurgie.* Bd. LXXIII., Heft 2, p. 347. 1904.

‡ A. E. Wright and Stewart R. Douglas. An Experimental Investigation of the *Rôle* of the Blood Fluids in Connection with Phagocytosis. Proceedings of the Royal Society. Vol. LXXII., No. 483, p. 357. October 31, 1903. *Ibid.* Vol. LXXIII., No. 490, p. 128. February and March, 1904.

the bacteria for the phagocytes. These substances are developed in the blood under certain conditions. Thus, injections of dead cultures of the S. pyogenes aureus into the blood produces a substance which prepares the live staphylococcus as food for the phagocytes. Substances acting in this way they call "opsonins" (*opsono*, I prepare victuals for).

These authors find that phagocytosis for certain organisms depends upon the presence of opsonins in the blood. Thus, B. typhosus, S. choleræ Asiaticæ, B. coli communis, B. dysenteriæ, S. pyogenes aureus, B. pestis, M. melitensis, D. pneumoniæ, are all taken up by phagocytes after being prepared by the opsonins. B. diphtheriæ and B. xerosis are not acted upon by opsonins.

Neufeld and Rimpau* found that antistreptococcus serum acts as an opsonin.

Just the contrary effect to opsonins, on the other hand, is produced by certain other bodies; for Bail† has found that when tubercle bacilli undergo bacteriolysis certain substances are liberated which check phagocytosis. These he regards as endotoxins, and gives to them the name *aggressins*.

There can be no doubt but that phagocytosis plays an important part in combating bacterial infection. And what follows in regard to the germicidal and antitoxic action of the fluid portion of the blood containing no phagocytes does not alter the fact that phagocytes, when these are present, do destroy the bacteria. Moreover, Metchnikoff maintains that the germicidal property of the blood-serum free from cells is due to substances liberated from the phagocytes by phagolysis, or breaking up of phagocytes. In other words, he holds that infection is combated by the phagocytes or by substances derived from the phagocytes.

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* *Deutsche medicinische Wochenschrift.* 1904. Bd. XXX., p. 1458.

† Oskar Bail. *Wiener klinische Wochenschrift.* Bd. XVIII. 1905. Review in *Bulletin de l'Institut Pasteur.* III. p. 348. 1905.

But be this as it may, it is well established that the serum of the blood deprived of leukocytes also has the property of destroying bacteria in many cases. It has been found that the serum of animals which have been inoculated with increasing amounts of culture is greatly enhanced in bactericidal power. Whether this enhanced power is due to substances liberated by the leukocytes or whether it is derived from other sources is a matter of dispute. It is not yet certain, in other words, what is the source of bacteriolysin, but its production is stimulated by the gradual inoculation of the animal with cultures.

It has already been explained that the neutralization of bacterial poisons or toxins takes place by the production of antitoxins (see page 176), and that the gradual injection of toxins is followed by a greatly increased production of antitoxin. Bacteriolysins, then, are produced—or at least their production stimulated—by injections of bacteria, antitoxins by injections of toxins; and Ehrlich advanced his now celebrated side-chain theory to explain these phenomena as well as the formation of agglutinins, lysins and precipitins.

Ehrlich's Side-chain Theory of Immunity.*—Ehrlich was the first to offer an explanation from a chemical point of view of the action of toxins on cellular protoplasm and the formation of antitoxins. He assumes, to begin with, that the molecules of the protoplasm are to be regarded as being endowed with chemical groups, present in the form of lateral appendages to the molecule, called side-chains. They can be illustrated

* The literature of this subject is very extensive. An exhaustive review is that by L. Aschoff. Ehrlich's Seitenkettentheorie. *Zeitschrift für allgemeine Physiologie.* 1902.

The following are also of a general character: Ehrlich's Croonian Lecture. Proceedings of the Royal Society. LXII., p. 437. 1900. Ehrlich. Schlussbetractungen. Nothnagel's System of Medicine. Vol. VIII. H. C. Ernst. Modern Theories of Bacterial Immunity. 1903. Prudden. *Medical Record.* February 14, 1903. Ritchie. *Journal of Hygiene.* Vol. II. 1902. Bergey. *American Medicine.* October 11, 1902. Immunity. Special Article. *Journal of the American Medical Association.* No. 4 *et seq.* 1905.

by the analogies presented by the graphically written formulæ of some complex molecules. It is necessary to conceive of molecules made of an immense number of atoms, and bristling with projecting side-chains. The function of the side-chains is to become attached to other organic molecules with which they have affinities. In this manner they aid in absorbing the substances essential for the nutrition of the protoplasm of cells.

The side-chains are therefore also called "*receptors*"—a more appropriate name. The numerous receptors which a molecule has are of many kinds, with affinities for other molecules of different kinds. Each kind of receptor will then have an affinity for a molecule of a particular kind, which it may be said to "fit," as a key fits in a lock, although this expression must not be taken in a literal sense. A receptor to which tetanus toxin might become attached would not "fit" diphtheria toxin. In order that toxins may be able to combine with the receptors their structure must be nearly like that of the food molecules which the receptors are adapted to receive.

Secondly, soluble toxins are to be looked upon as definite chemical bodies excreted by bacteria, and containing two essential groups of atoms. One group is the *haptophore*, by means of which the toxin may be linked with the receptors of the molecules of the cell. The other group is the *toxophore*, which is capable of destroying the protoplasmic molecule, after being attached to the receptor of the latter by the haptophore.

These relations have been represented schematically. In Fig. 48 a portion of a cell is shown, with receptors. A molecule of toxin, *b*, is attached by its haptophore, *c*, to the haptophore of the cell receptor, *a*. A free cell receptor is also shown with its haptophore, *e*, capable of uniting with any toxin molecule that may be present. The toxin molecule, *b*, has its toxophore group represented by the fringe-like end, *d*. If the cell receptor becomes detached from the cell, its haptophore, *e*, may unite

with a toxin molecule as readily as when the receptor is still attached to the cell. Such a detached receptor constitutes a molecule of antitoxin.

As the side-chains or receptors of the protoplasm are essential to its existence, their combination with the toxin, through its haptophore, results in destruction of the molecule. But if the damage be not too serious, the protoplasm is stimulated to produce numerous similar side-chain groups—to an overproduction of these, in fact. As not all of these are necessary for the performance of its functions, the superfluous ones are thrown off into the surrounding serum. It is well known that many cells of the body exhibit analogous heightened activities under stimulating influences, as pointed out by Weigert. If such free side-chains or receptors combine with the haptophorous groups of the toxin, the latter is no longer able to combine with the protoplasm of the cells. Thus they act as a kind of buffer in protecting the protoplasm from the attacks of the toxins. These free, cast-off receptors constitute the antitoxic part of the serum as stated.

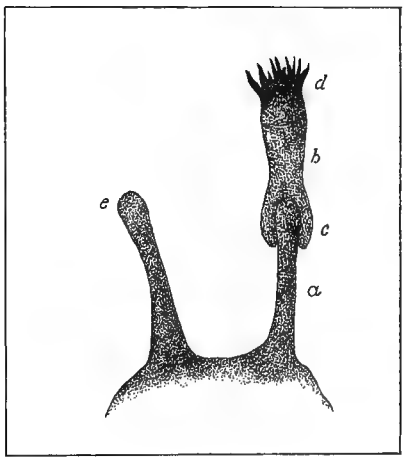

FIG. 48.—RECEPTORS OF THE FIRST ORDER UNITING WITH TOXIN.—(*Journal of the American Medical Association.* 1905. P. 955.)

a. Cell receptor. *b*. Toxin molecule. *c*. Haptophore of the toxin molecule. *d*. Toxophore of the toxin molecule. *e*. Haptophore of the cell receptor.

Numerous experiments have been made which illustrate the probable chemical nature of antitoxin action. A fatal

dose of diphtheria or tetanus toxin may be neutralized outside of the body by mixing it with its appropriate antitoxin. Injection of the mixture shows it to be innocuous to animals.

The manner in which toxins combine with protoplasm has been shown in the case of tetanus toxin. The filtrate from cultures of tetanus bacilli will kill guinea-pigs, presumably by damage to the central nervous system. The same filtrate rubbed up with brain or spinal cord has been found to have lost its toxic properties. It may be assumed that the poison has combined with the protoplasm of the cells.

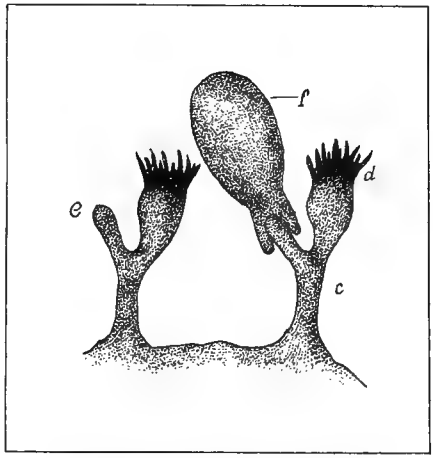

FIG. 49.—RECEPTORS OF THE SECOND ORDER AND OF SOME SUBSTANCE UNITING WITH ONE OF THEM.—(*Journal of the American Medical Association.* 1905. P. 1113.)

c. Cell receptor of the second order. *d.* Toxophore or zymophore group of the receptor. *e.* Haptophore of the receptor. *f.* Food substance or product of bacterial disintegration uniting with the haptophore of the cell receptor.

But the side-chain theory offers an explanation not only of the mechanism of the union of toxin and antitoxin, but also explains the phenomena of agglutination, precipitation, and cytolysis. In the union of antitoxin and toxin, as stated above, the process is a simple combining of the toxin with the receptor, and there the process ends. Receptors of this kind are called receptors of the first order (Fig. 48). But after the union of the agglutinins and of the precipitins with their receptors further change takes place. In the one case, clumping; in the other, precipitation; and these changes are

brought about by a kind of fermentative action. So in addition to the haptophore group the receptor must possess a ferment-producing group. It seizes on the red cells or on the bacteria, as the case may be, with the haptophore group, and produces certain changes with its ferment-producing group. The latter is called the zymophore group. Receptors of this kind are called receptors of the second order (Fig. 49).

With the lysins there is also a change, which takes place after the receptor unites with the bacteria or other cells; so there must be here also a zymophore or zymotoxic group, as it is called. This zymotoxic group, however, is not an integral part of the receptor, but is easily broken off from it, in the manner described below.

As is explained later, the power of the lysins becomes suspended, but not lost, on being heated to 55° or 56° C. In this condition they are said to be inactivated. They become active again when certain fresh serum is added—not necessarily fresh lysin, but fresh normal serum. This will all be discussed and explained later. For the present purpose it is sufficient to bear in mind that lysin becomes inactivated and may be reactivated. So the lysins act differently from agglutinins and precipitins. They must have peculiar receptors which unite, on the one hand, with the cells which they disintegrate, and, on the other, with a ferment-producing substance easily destroyed by heat. These receptors must possess two haptophore groups; in other words, a haptophore for cells and a haptophore capable of uniting with a body containing a ferment-producing group. Receptors for lysins are therefore called amboceptors, or receptors with two haptophores (Fig. 50). They are also called receptors of the third order. The substance which reactivates the lysin, the fresh serum, is called complement, and it must be composed of a haptophore in order to attach itself to the amboceptor, and a zymotoxic group in order to produce lysis. On heating fresh normal serum to 55° or 56° C. the complement

which it contains is not destroyed, but its zymotoxic group alone is destroyed; the haptophore group, on the other hand, resists heat. So if heated complement be added to inactivated lysin, it unites with the freed haptophore. A lysin inactivated by heat with fresh serum added disintegrates homologous cells; but a lysin inactivated by heat when heated fresh serum is added will not only not produce lysis of homologous cells, but will not do so even when unheated fresh serum is subsequently added.

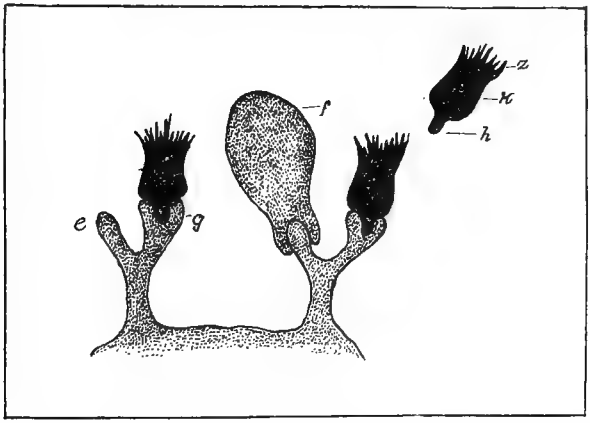

FIG. 50.—RECEPTOR OF THE THIRD ORDER, AND OF SOME SUBSTANCE UNITING WITH ONE OF THEM.—(*Journal of the American Medical Association.* 1905. P. 1369.)

c. Cell receptor of the third order—an amboceptor. *e*. One of the haptophores of the amboceptor, with which some food substance or product of bacterial disintegration (*f*) may unite. *g*. The other haptophore of the amboceptor with which complement may unite. *k*. Complement. *h*. The haptophore. *z*. The zymotoxic group of complements.

The behavior of mixtures of toxins and antitoxins is most peculiar, for they do not in all cases obey the simple rule of relative proportion. It is true that if a certain amount of antitoxin neutralizes a certain amount of toxin, then any multiple of this amount of antitoxin will neutralize the same multiple of toxin if the two are mixed all at once. So far the rule is simple. But if 100 doses of toxin—*i. e.*, enough to kill 100 guinea-pigs—is exactly neutralized, and then the amount of free toxin necessary to kill a guinea-pig is added, it will not kill a guinea-pig as would be expected. Many doses have to be added, sometimes as much as thirty or forty doses or more, before the mixture again becomes poisonous.

Another remarkable property is that toxin that has stood for a long time loses greatly in poisonous properties, but not in its power of combining with antitoxin. Furthermore, this old toxin will produce antitoxin if injected into horses or other susceptible animals.

In order to explain these extraordinary reactions several theories have been advanced, and in this connection certain peculiar reactions obtained with lysins, agglutinins, and precipitins, which have helped to give an insight, have also been explained on similar theoretical grounds. These theories will now be discussed.

Ehrlich regards the beef-broth from a diphtheria or tetanus culture as a solution of several different but related bodies, and he makes so-called "spectra" to explain this idea. He thinks that primarily the substances are toxin and toxon (Fig. 51), each having affinity for antitoxin; but the affinity of toxon for antitoxin is weaker than the affinity of toxin for the antitoxin. And, furthermore, toxon—no matter in what dose—does not kill guinea-pigs quickly if at all, but causes a paralysis of the animal after some weeks of incubation, while

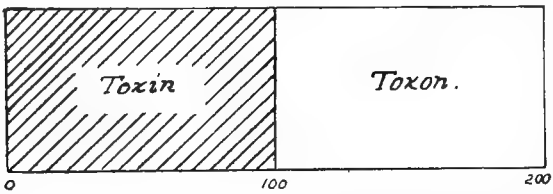

FIG. 51.—"SPECTRUM" OF THEORETICALLY FRESH, CRUDE TOXIN. Such a combination probably does not occur.

toxin, on the other hand, in just the proper amount kills a guinea-pig weighing 250 grams in two days. This is the standard minimum fatal dose, or 1 d. l. (dosis letalis).

Now, if enough antitoxin is added to the poisonous beef-broth it will neutralize both the toxin and the toxon, but if not enough is added for this, the toxin is first neutralized and the toxon still produces paralysis of the guinea-pig.

But on standing, both toxin and toxon quickly become changed; a part of the toxin is converted into a body called toxoid and a part of the toxon into toxonoid, and, while retaining their affinity for antitoxin, these are both weakened in pathogenic power as compared with the original toxin; toxoid, in fact, is devoid of toxic properties. Toxoid, then, is toxin deprived of its toxophore, but it retains the haptophore group. Still further, the resulting toxoid and the toxon have each three different degrees of affinity for antitoxin.

A part of the toxoid has less affinity than the toxin; a part equal; a part more. These are designated epitoxoids, syntoxoids and protoxoids, respectively (Figs. 52 and 53), and toxons are in like manner designated epitoxons,

syntoxons and protoxons. But, since all toxons have less affinity for antitoxin than toxin has, it follows that epitoxoid and toxon are the same. All crude toxin, then, is composed of a mixture of toxin, toxoid and toxon, for toxoids begin to form immediately, so that toxin-toxon without toxoid is not known.

When enough antitoxin is added to 100 doses (100 d. l.'s) of crude toxin to just neutralize it, all the toxin and all the toxon are united to antitoxin. But if fresh toxin is added, some of the toxoid and toxon is liberated, and the added toxin becomes attached to the antitoxin in its place; and so with each additional

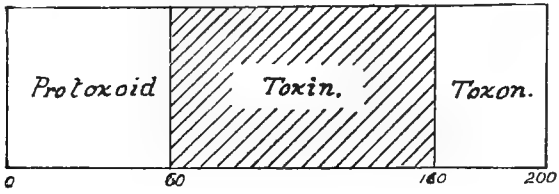

FIG. 52.—"SPECTRUM" OF VERY FRESH CRUDE TOXIN.

amount of toxin added more toxon and toxoid is liberated till the point is reached where all the toxon and toxoid is free, and the additional toxin finds all the haptophores of antitoxin occupied by the toxin previously added. In this case any additional toxin remains uncombined, and, if such a mixture is injected into a guinea-pig, the animal is killed.

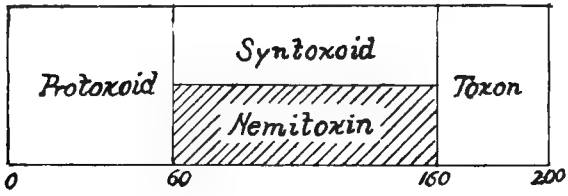

FIG. 53.—"SPECTRUM" OF CRUDE TOXIN AS IT IS SUPPOSED PRACTICALLY ALWAYS TO OCCUR.

Bordet's* explanation differs from Ehrlich's. Bordet does not admit the existence of toxons, and regards the paralysis attributed by Ehrlich to the action of this hypothetical substance as due to weakened toxin. He explains the peculiar behavior of a neutralized mixture of crude toxin with antitoxin, stated above, by assuming that antitoxin is capable of taking up and neutralizing varying amounts of toxin. He compares the effect of mixing toxin and antitoxin to that of mixing starch and iodine: the more iodine added to the starch,

* Bordet. Toxines et Antitoxines. *Annales de l'Institut Pasteur.* 1903. Pp. 161 *et seq.*

the bluer the color. Let A represent, then, a certain amount of antitoxin; let A be capable of combining 1, 2, 3, 4, 5, different amounts of toxin; call these amounts of toxin T_1, T_2, T_3, T_4, T_5; and assume that a combination in which all the A's are combined with T's in the proportion of AT_1, is neutral, that it has no poisonous properties; that a combination represented by AT_2 also has no toxic properties, but that AT_3 would begin to show toxic properties, and that AT_4 is distinctly toxic, and that AT_5 is very toxic. Starting with toxin, then, if just enough antitoxin is added to neutralize its poisonous properties, AT_1 is first formed, which is not toxic; now add more toxin, and none of this remains free, but, on the contrary, AT_2 is formed, which is not toxic; on adding still more, when AT_4 or AT_5 is reached the mixture is fatal for guinea-pigs. The paralysis which Ehrlich attributes to toxon would be represented, say, by AT_3. In between the combinations represented by AT_1 to AT_5 are all imaginable combinations, a sliding scale of no definite units.

In other words, while Ehrlich holds that toxin and antitoxin unite in one definite proportion, Bordet holds that they may unite in any proportions, like two different colors of paint mixed together producing any intermediate color with more or less tint of one or other of the two original colors.

The evidence adduced by Bordet for this conception is very abundant and fully repays study.

Still another theory offered to explain the peculiar behavior of the antitoxin-toxin mixture is advanced by Arrhenius and Madsen, also supported by experimental evidence. They also deny the existence of toxon, and look upon a mixture of antitoxin and toxin as analogous to an amphoteric mixture of a dilute acid and alkali, or of an acid and alcohol. In such combinations there are compounds formed of the two substances, but some of each of the two constituents remains free. An ester is not only a compound formed by an acid and an alcohol, but it has free alcohol and free acid. Moreover, the ester is constantly changing, some of the alcohol and some of the acid separating and new ester constantly forming again. When first mixed, more ester is formed, and less alcohol and acid are liberated, till a point of dynamic equilibrium is reached, when just as much ester is formed as there are alcohol and acid liberated. Just so in adding toxin to antitoxin: at first more of the two combine than is set free, but after a time a condition of dynamic equilibrium is established, and any additional toxin remains free.

Briefly stated, these are the three theories which are now advanced by competent authorities, and, if these outlines are kept clearly in mind, it will not be difficult to understand the subject as presented in the many medical journals and the many monographs which have appeared on the subject.*

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* A summary will be found in a monograph by Michaelis. Die Bindungsgesetze von Toxin und Antitoxin. Berlin. 1905. Also a special article entitled, Immunity. *Journal of the American Medical Association.* Nos. 4 *et seq.* 1905.

The theories of Ehrlich and Bordet in regard to the composition of lysins may also be appropriately discussed in this connection, as it is from the studies of these bodies that many of the ideas in regard to immunity have been developed.

A lysin contains two substances, a thermolabile and a thermostabile substance—*i. e.*, one readily destroyed by heating at 55° C. for a half-hour, the other resisting much higher temperatures. The thermostabile substance is now called by Ehrlich the *immune body*, the thermolabile the *complement*, though Ehrlich has used in the past various other names for these hypothetical bodies. Bordet uses the name *substance sensibilisatrice* for the thermostabile, Ehrlich's immune body, and *alexin* for the thermolabile or Ehrlich's complement. Both are agreed that there are two bodies concerned; both are agreed as to the property of the one to be readily destroyed by heat, of the other to resist heat.

In English writing it is more common to use the German than the French terms, so these will be employed, though a great deal of what is known about lysins has been contributed by Bordet and the French school generally. The word alexin was first used by Buchner, but is used now mostly in French writings.

It should be recalled that a lysin is the substance formed in the blood-serum of an animal when the latter is injected with bacteria or with foreign red blood-cells. A rabbit injected with typhoid bacilli develops lysin for typhoid bacilli; when injected with red blood-cells of a guinea-pig, develops a lysin for guinea-pig red cells.

If lysin is heated to 55° C. for thirty minutes, it loses its complement (or *alexin*) and the immune body (*substance sensibilisatrice*) only remains; so that red cells which are disintegrated by the unheated lysin remain intact in the heated lysin. But the heated lysin becomes active again if either fresh unheated rabbit's or guinea-pig's serum is added to it. The heated lysin is spoken of as inactivated; the heated lysin with fresh serum, reactivated. The fresh serum which is added contains the complement (*alexin*); the heated lysin contains only the immune body (*substance sensibilisatrice*).

The immune body is specific, but the complement is not; at least the blood of some animals contains complements for several different immune bodies. Thus, fresh horse serum added to various inactivated lysins reactivates the latter. But chicken blood-serum does not contain complement for chicken corpuscles. For if chicken hemolysin, produced by injecting a rabbit with chicken red cells, is heated to 55° C. for thirty minutes (inactivated), it will not disintegrate chicken red cells if fresh chicken serum be added; but if fresh rabbit serum is added, it will hemolize chicken red cells as it did before heating.

The immune body becomes fixed to the red cells, as can be shown by adding red cells to inactivated lysin and then washing these with salt solution. If after adding the red cells to the inactivated lysin the mixture is centrifugalized and the precipitated red cells washed with salt solution so as to remove all of

the free immune body, the precipitated, washed red cells disintegrate when fresh complement—*i. e.*, fresh serum—is added.

From these and other considerations Bordet regards lysin as composed of a specific antibody, *sensibilisatrice* or immune body of Ehrlich, on the one hand, and of a cytolytic, bacteriolytic, hemolytic alexin proper or complement of Ehrlich, on the other. The immune body is specific, but it does not cause destruction of cells by itself: it does so only in conjunction with complement or alexin. Alexin is not strictly specific, and it has some cytolytic power, as seen in normal blood independently of *substance sensibilisatrice*. But its power is greatly enhanced if the cells acted upon are first sensitized by *sensibilisatrice*.

The reactions found by Bordet may be briefly summarized as follows:

Bacteria or other cells which are united to the same immune body or *sensibilisatrice* become disintegrated upon the addition of diverse complements.

In any one cytolytic serum the complement for bacteria and for red cells is one and the same. The fixation of the immune body takes place by the stroma of the red cells, for red cells washed of all their contents so that only the empty capsules are left fix the immune body just as well as unwashed red cells.

Antilysin combines with both immune body and complement.

Since antilysin neutralizes the complement, it is both antihemolytic and antibacteriolytic, because the complement for both of these is the same.

Pfeiffer was the first to describe the bacteriolytic action of animal fluids on bacteria, and the reaction is called Pfeiffer's phenomenon. Pfeiffer's view of the nature of lysin differs from those of Ehrlich and of Bordet. He holds that lysin is not composed of two bodies, but that it is one body which is readily changed into an active and passive condition. This view does not seem to have found as much favor as that of Ehrlich and that of Bordet.

CHAPTER VIII.

DISINFECTANTS AND ANTISEPTICS.*

A **disinfectant,** strictly speaking, is a substance capable of killing bacteria, but the term is now rather loosely applied to substances which inhibit the growth, or merely destroy or disguise, disagreeable odors. It is mainly used for substances employed for sterilizing rooms, cars and the like.

Sterilization is the term employed for the process of destroying bacteria by heat or other means.

A **germicide,** as its name implies, is an agent which kills bacteria, and it is restricted to this meaning.

An **antiseptic** is a substance capable of preventing the growth and reproduction of bacteria. It differs from a germicide in that it simply prevents development without actually killing the bacteria.

A **deodorizer** is a substance capable of so changing a noxious odor that it is less unpleasant. At the present time the term is usually and properly restricted to those substances which, without germicidal action, simply disguise or destroy an odor.

Testing Disinfectants and Antiseptics.

The determination of the antiseptic value of a material is a comparatively simple matter. A virulent culture of the organism used as a test is inoculated into sterile bouillon containing a known quantity of the antiseptic. The process is repeated with varying strengths of the material until the smallest

* By Thomas B. Carpenter, M.D., Assistant City Bacteriologist, Buffalo, N. Y.

quantity of it capable of preventing growth is determined. This dilution may be considered the antiseptic value of the material in question for the organism used, under the conditions of the test.

Determination of the germicidal power of a substance is a problem of greater difficulty, and the method used must be adapted to the requirements in each case. It is obvious that some of the substance tested remains in contact with the organisms in the method given for determining the antiseptic value, and that we do not know whether the bacteria are alive and merely inhibited in growth, or actually killed in such tests.

Sternberg's Method.—To a measured quantity of a virulent bouillon-culture of the test-organism is added a given amount of the germicide. After varying lengths of time inoculations are made from this mixture into culture-media, preferably bouillon, and note is made of the presence or absence of growth under suitable conditions of temperature and the like. The shortest exposure to the weakest solution of the substance necessary to kill the test-organism is taken as the germicidal value of that substance for the particular organism used.

Koch's Method.—This method is usually employed for spore-bearing bacteria, like the bacillus of anthrax. The hay bacillus is convenient to use when experiments are being made by large classes of students. Small pieces of sterile silk or cotton thread are soaked for some hours in a bouillon-culture of the test-organisms. The threads are then removed, partially dried and placed in a solution of known strength of the germicide, and exposed for a definite length of time. The thread is removed from the solution, washed carefully in sterile water, dropped into a tube of sterile bouillon plugged with cotton, and growth or absence of growth noted. As in other methods, the greatest dilution of the germicide that will kill the test-organism in the shortest time is taken as the germicidal value of that substance for the organism used.

Hill's Method.—The test-organism is dried upon the end of a sterile glass rod contained in a sterile test-tube, the end of the rod projecting through a cotton plug. The end of a glass rod is immersed in a fluid culture of the test-organism and allowed to dry. While drying it is inserted into a sterile test-tube, and plugged around with cotton. It is then ready to test by exposure to any germicide, either liquid or gaseous. After exposure to the germicide it is plunged into a tube of sterile beef-broth in order to see whether the organisms adhering are all killed.

All of these methods are open to serious sources of error, particularly in the testing of powerful germicides. In Sternberg's method, small quantities of the substances tested may be carried over with the organisms, and, if a powerful germicide, in sufficient amount to prevent growth, and thus give erroneous results. In Koch's or Hill's method this factor may be partially obviated by washing in sterile water after exposure to the germicide. This does not remove another source of error, namely, the chemical action that may take place between the substance and the protoplasmic contents of the bacterial cell. This action may extend deeply enough to restrain the growth of an organism for a very long time without actually killing it. When placed under suitable conditions, such union may be broken up and the organism regain its power to develop. It has been suggested that, to remove errors in the above methods, the bacteria after exposure to the germicide be inoculated into susceptible animals; but Sternberg's experiments in this direction have shown that bacteria may become so attenuated in virulence by the action of germicides insufficient to kill that the value of animal inoculation experiments is limited. Moreover, it sometimes happens that it is desired to test germicides on bacteria which are not pathogenic for animals.

Geppert suggested a valuable modification of these methods while determining the germicidal value of bichloride of mer-

cury. After exposing his test-organism to bichloride of mercury, and before inoculating into bouillon to determine death of the organism, he treated with a dilute ammonium sulphide solution, thus effectually neutralizing any mercury-salt remaining.

Sedgwick developed this method still further, and to him we are indebted for demonstrating its accuracy and practicability.

Sedgwick's Method.—To 15 c.c. of sterile water in a 60 c.c. Erlenmeyer flask add 2 c.c. of a virulent culture of the test-organism. Then add a solution of the substance under investigation in the proportion necessary to give the dilution wished. Mix thoroughly, and allow this "action-flask" to stand as long as it is desired to have the germicide in contact with the test-organism (action-period). Transfer 0.5 c.c. from the action-flask to a flask containing 200 c.c. of a solution of some chemical capable of decomposing the substance being tested with the formation of inert or insoluble compounds. In this "inhibition-flask" the strength of the solution should be such that molecular proportions of the chemical are present in sufficient quantity to combine with all the germicides carried over. The inhibition-flask is shaken for 30 seconds, and 1 c.c. transferred from it to 100 c.c. of sterile water in another, the "dilution-flask." After two minutes, three agar tubes are inoculated with 1 c.c. each from the dilution-flask, plated, and growth watched for.

Control-experiments should be performed to determine that the dilution of the test-culture is not too great when carried through the three flasks. It likewise should be determined that the inhibiting chemical itself has no injurious effect on the bacteria.

The inhibiting chemical must be determined for each individual case. For salts of the heavy metals ammonium sulphide answers well; for mercury salts, stannous chloride

may be used; for formaldehyde, ammonium hydrate; for carbolic acid, sodium sulphate.

The testing of gaseous disinfectants, such as sulphur dioxid and formaldehyde, should be conducted under conditions as nearly identical with those met with in actual practice as possible. The test-organisms may be exposed on threads or glass rods, and acted upon by a known volume of strength of germicide for a known length of time. Subsequent treatment of the organisms with a suitable inhibitor is necessary when possible, and should growth occur in the cultures following, the test-organism should be identified in order to exclude possible contamination by extraneous organisms.

In determining the value of germicides for sterilizing ligatures, the students can apply methods based on the foregoing principles. Great care is necessary to arrive at correct conclusions, particularly in the case of animal tendons. In many instances quite stable compounds are formed between tendon and germicide, and living organisms may be so imbedded in such a substance that subsequent growth in a test-culture is impossible. The use of a suitable inhibitor, and, prior to final culture-tests, a prolonged soaking in sterile water, will promote the accuracy of the results.

So many and often such obscure chemical and physical factors enter into the action of chemical germicides that uniform results are not possible within narrow limits. This accounts for the conflicting results obtained by different investigators, and even by the same investigator at different times. A number of variable and only partially controllable conditions enter into every test. Results with gaseous disinfectants are especially uncertain on this account. None of them have any great power of penetration, and consequently act only where the bacteria are freely exposed and then not always with certainty.

Chemical Disinfection.*

Heat properly applied is the simplest and at the same time the surest disinfectant (see Part I., Chapter II.); but for many purposes it cannot be used, and we have recourse to those chemicals that practice and investigation have shown to be of value. *The efficiency of chemical disinfectants as ordinarily used is overrated.* An immense number of substances possess germicidal properties, but, unfortunately, the majority are objectionable in that they are expensive, intensely poisonous, or so corrosive that damage may be done to articles of value with which they may come in contact.

In the following pages only those substances which are in common use or seem to be of special value will be considered.

Mercuric Chloride or Corrosive Sublimate.—This substance is probably more commonly used than any other one germicide. In the strength of 1–1000 it will sometimes kill the spores of anthrax within a few minutes (see Bacillus anthracis, Part IV.). It is claimed that its affinity for albuminous bodies and the readiness with which it combines with such substances detract from its value for some purposes. On the other hand, many observers claim that the albuminous combinations formed under such circumstances are soluble in an excess of albuminous fluid, and that its value as a germicide is not affected thereby. To obviate this possible difficulty it is customary in practice to combine the bichloride of mercury with some substance that will prevent the precipitation of the mercury salt by albumin. For this purpose 5 parts of any one of the following substances to 1 part of bichloride of mercury may be used—hydrochloric acid, tartaric acid, sodium chloride, potas-

* For fuller details on this subject consult Rosenau. Disinfection and Disinfectants. 1902.

sium chloride, or ammonium chloride. A very practical stock-solution for laboratory purposes has the following composition:

Hydrochloric acid	100 c.c.
Bichloride of mercury	20 grams.

Five c.c. in a liter of water makes a solution of about 1–1000 strength.

Mercuric Iodide.—An extremely high antiseptic value has been placed on this substance by Miquel, who claims that the most resistant spores are prevented from developing in a culture-medium containing 1–40,000. In combination, as potassio-mercuric iodide, it has been used in soaps (McClintock) with very favorable results. The substance is not extensively employed, and further investigation is necessary to determine its true value.

Attempts are being made to manufacture combinations of mercury and other powerful metallic germicides with organic acid and basic bodies, the purpose being to utilize the metallic base in greater strength without injury to the living tissues. Such compounds are exemplified by *mercurol*, said to be a combination of mercury with nucleinic acid, and to possess active germicidal properties, great penetrating power, and no injurious effect on living tissue. It is also said to have a particularly destructive action upon the gonococcus.

Silver Nitrate.—This salt probably occupies the next position to the bichloride of mercury in germicidal power. Behring claims it to be superior to bichloride of mercury in albuminous fluids. The anthrax bacillus is killed by a solution of 1–20,000 after two hours' exposure. At least forty-eight hours' exposure to a 1–10,000 solution is required to kill the spores of anthrax. It is very irritating, and possesses strong affinities for chlorides, forming with them insoluble chloride of silver, a salt without germicidal value. For these reasons the use of silver nitrate is limited. In the solutions usually employed for douching the cavities of the body the available silver nitrate is immediately

converted into the insoluble chloride, and little if any germicidal action takes place. To this fact may be ascribed the varying clinical results reported.

Many semi-proprietary silver compounds are on the market, introduced to replace the nitrate and its objectionable features. The most important are argentamin, argonin, protargol and argyrol, all organic silver combinations. They do not combine with chlorides, are less irritating than the nitrate, and, not coagulating albumin, they possess greater penetrating power. Clinical reports and investigations have been so contradictory thus far that their value cannot be readily estimated.

Carbolic Acid.—One of the most important and most widely used germicides. It is usually employed in strengths of from 1 to 5 per cent. A 3 per cent. solution will sometimes kill the spores of anthrax after two days' exposure (see Bacillus anthracis, Part IV.). In the absence of spores the anthrax bacillus is destroyed by a 1 per cent. solution in one hour. The less resistant pus cocci are destroyed rapidly by a 2 per cent. solution. Combination with an equal proportion of hydrochloric acid enhances the efficacy of carbolic acid to a marked extent. This is due to the prevention of albuminous combinations, thus allowing greater penetration of the germicide.

Many other substances closely related to carbolic acid are used and possess marked germicidal properties. Among them may be mentioned creolin, cresol and lysol. They are all slightly superior to carbolic acid in actual germicidal value.

Aniline Dyes.—Many of these substances, notably pyoktanin (methyl-violet), possess germicidal properties. A solution of 1–5000 will kill the anthrax bacillus in two hours. A much stronger solution, 1–150, is required to kill the typhoid bacillus in the same time. Malachite-green is said to possess even greater germicidal value than pyoktanin. Methylene-blue also possesses considerable germicidal power.

Formalin is a 40 per cent. aqueous solution of formaldehyde.

Results of the earlier investigations seemed to show that formaldehyde possessed remarkably high germicidal properties, but later experiments have failed to corroborate these. In solutions of 1–1000 an exposure of twenty-four hours is necessary to destroy the Staphylococcus pyogenes aureus, while 1–5000 is sufficient to restrain its growth (Slater and Rideal). Its use in a gaseous form as a house-disinfectant is by far the most important application at the present time.

Harrington's investigations have shown that an atmosphere produced by vaporizing 435 c.c. of formalin (40 per cent. aqueous solution of the gas) in 1000 cubic feet of air space, equivalent to 1 quart to a room 15 feet square and 10 feet high, will destroy all exposed organisms in half an hour; when protected by one fold of cotton-cloth, an exposure of one and one-half hours is necessary. In a perfectly dry atmosphere the gas penetrates slightly, and will disinfect through one layer of cotton-cloth; in a moist atmosphere no penetration can be obtained.

In vaporizing the gas many methods have been employed. Simple evaporization of solutions without heat cannot be relied upon, for the solid, polymerized paraformaldehyde is easily formed under these circumstances. Better results can be obtained with the aid of heat, although polymerization is apt to occur unless evaporation is rapid. To produce the best results it has been found necessary to use special forms of lamps or generators for its production, a few of which are mentioned below.

Sanitary Construction Company's Lamp.—This lamp consists of a tank to hold the formaldehyde solution, and a spiral tube by which the solution is slowly conducted through a flame and vaporized. The necessary amount of solution is placed in the tank and the apparatus started, outside the room, the gas being conducted through the keyhole by a suitable tube.

Trillat Autoclave.—This apparatus consists of a small silver-lined pressure-boiler, fitted with lamp, safety-valve, pressure-gauge, thermometer, and escapement-tube. The necessary amount of formaldehyde solution is placed within the apparatus,

together with an equal amount of 20 per cent. solution of calcium chloride; the addition of the latter salt is to prevent formation of the solid polymeric modification, the so-called paraform. The autoclave is closed and heated from below to a temperature of 135° C. The escapement-valve is then opened carefully and the gas allowed to enter the room slowly through the escapement-tube, which has meanwhile been passed through the keyhole. About thirty minutes are required to discharge all the gas from 500 c.c. of solution. If the temperature has not been allowed to go above 135° C., the gas will contain but little moisture and possess its maximum efficiency.

Schering Lamp.—In this form of lamp formaldehyde is generated by the decomposition of paraform or paraformaldehyde, a polymeric modification of formaldehyde, occurring as a white salt. It is decomposed by heat, giving off formaldehyde gas. It is placed on the market in the form of tablets, each one of which yields a definite amount of gas. The lamp consists of a small iron tray for the reception of tablets, and so arranged above the heating apparatus that sufficient draught is created to carry off the gas as rapidly as formed. In operating, a sufficient number of tablets are placed on the tray, the lamp lighted and placed in the room to be disinfected.

Methyl-alcohol Lamps.—Several of these lamps are on the market, all operating on the well-known principle of the oxidation of wood-alcohol to formaldehyde when the alcohol is vaporized by projection against a heated, platinized, asbestos disk. In operating such an apparatus, the alcohol is lighted until the asbestos disk becomes hot. The flame is then extinguished; the heat from the disk is sufficient to vaporize the alcohol, which undergoes oxidation and keeps the disk at a red heat. When the apparatus is operating in a satisfactory manner, the room is closed and disinfection allowed to proceed. It must be said, however, that it is difficult to estimate or control the amount of formaldehyde evolved in generators of this type.

Formaldehyde Candles.—Mixtures of paraformaldehyde and paraffin or other combustibles, which may be moulded into candles, each enclosed in a tin case, make a convenient apparatus to generate formaldehyde gas for room disinfection. The candle is placed in a suitable fireproof dish, it is then ignited, and generation of the gas is allowed to proceed in the tightly closed room.

Sulphur Dioxide.—This substance is used extensively for house disinfection, and is usually prepared by burning sulphur. Much difference of opinion exists regarding the value of it as a disinfectant. The spores of anthrax are not killed by several days' exposure to the liquefied gas. Anthrax and other bacilli are destroyed in thirty minutes when exposed on moist threads in an atmosphere containing one volume per centum of the gas. An exposure of twenty-four hours in an atmosphere containing four volumes per centum of the gas will destroy the organisms of typhoid fever, diphtheria, cholera and tuberculosis. The presence of moisture greatly enhances the activity of the disinfectant, owing to the formation of the more energetic sulphurous acid.

For the destruction of insects, such as mosquitoes, this agent is superior to formaldehyde. Its application for this purpose is important in preventing the spread of yellow fever and malaria.

In practice, at least 3 pounds of sulphur per 1000 cubic feet should be used, and moisture must be present. This latter requirement can be fulfilled by evaporating several quarts of water within the tightly closed room just prior to generating the gas. In using powdered or flowers of sulphur, the necessary amount is placed on a bed of sand or ashes in an iron pot, which should be supported on some bricks in a pan or other vessel containing an inch or two of water. The sulphur is ignited by means of some glowing coals, or by moistening with alcohol and applying a match. Difficulty is often experienced in keeping the

sulphur burning, and for this reason it is surer and more convenient to use the so-called sulphur candles now on the market. In operating with these, a sufficient number are placed on bricks in a pan of water and the wicks lighted. Liquefied sulphur dioxide may be used, and can now be obtained in convenient tin receptacles containing a sufficient quantity for the disinfection of an ordinary room. The can is opened by cutting through a soft metal tube projecting from the top. The fluid vaporizes at the room temperature, and it is simply necessary to place the can in a convenient porcelain dish and allow the fluid to evaporate.

Sulphur dioxide is objectionable on account of its lack of power when dry, and on account of its corrosive action on metal and its bleaching effect on hangings and draperies in the presence of moisture; it is, therefore, preferable to use formaldehyde when possible.

Chlorine.—A very active gaseous disinfectant, particularly in the presence of moisture. An atmosphere containing 1 per cent. of the dry gas is fatal to anthrax spores in three hours. The anthrax bacillus is killed in twenty-four hours by exposure to a moist atmosphere containing the gas in the proportion of 1–2500. The bacillus of tuberculosis is killed by an exposure of one hour to a moist atmosphere containing the gas in the proportion of 1–200. Extremely minute quantities in solution will prevent the development of putrefactive organisms. The substance has been used for house and ship disinfection, but is now seldom employed on account of its extremely irritating properties and the difficulty of handling.

Bromine.—Used in the gaseous and liquid form. The dry vapor possesses but little disinfectant power; when moist it is much more efficient. In saturated aqueous solution it will kill the anthrax bacillus in twenty-four hours.

Calcium Hypochlorite, usually known as Chloride of Lime.—This is a most practical and valuable disinfectant, depending

for its efficiency on the available chlorine contained in it. Its alkalinity favors penetration, and for many purposes it cannot be excelled. A 1 per cent. solution will destroy anthrax spores in one hour. A solution of the same strength will disinfect typhoid stools in ten minutes.

Lime.—The addition of 0.1 per cent. of unslaked lime to fluid-cultures of the typhoid bacillus and cholera spirillum will render them sterile in four or five hours. Typhoid dejecta are sterilized in six hours by the addition of 3 per cent. of slaked lime; the addition of 6 per cent. will accomplish the same result in two hours. A convenient form for practical use is an aqueous mixture containing 20 per cent. of lime—so-called milk of lime. Typhoid and cholera dejecta are sterilized in one hour after the addition of 20 per cent. of this mixture. In practice it is safer to use a considerable excess of lime. From the foregoing facts it would seem probable that lime or whitewash as ordinarily applied would possess disinfectant properties. Experimental work has demonstrated this to be a fact. The organisms of anthrax, glanders and the pus cocci were destroyed within twenty-four hours by one application. For spore-forming organisms and the bacillus of tuberculosis the power is not so great, the latter organism not being destroyed by three applications of the whitewash. This is due, perhaps, to the large amount of fatty matter in the bacillus of tuberculosis, and suggests the possibility of enhancing the efficacy of the lime by the addition of a small proportion of caustic alkali.

Hydrogen Peroxide.—This substance is placed on the market in solutions varying in strength from 10 to 30 volumes; the mode of expression indicating that corresponding solutions will liberate ten to thirty times their volume of oxygen when appropriately treated. It possesses the property of rapidly oxidizing purulent secretions, and on this account is much used for cleansing infected wounds. It deteriorates in strength

so rapidly that only fresh solutions of known strength should be used.

Potassium Permanganate.—Koch asserts that a 3 per cent. solution will destroy anthrax spores in twenty-four hours, but that a 1 per cent. solution cannot be depended upon to kill pathogenic organisms. Its disinfectant value in practice is very low on account of its ready decomposition by inert material. In the dilute solutions usually used for medicinal injections and irrigations no disinfectant action occurs.

Iodoform.—This substance possesses little if any disinfectant power. It is mildly antiseptic in moist wounds, due to the gradual liberation of small quantities of iodine.

Boric Acid.—This material possesses practically no disinfectant power. It is a mild antiseptic when applied as an undiluted powder to wounds. A saturated aqueous solution is much used, and is weakly antiseptic.

Essential Oils.—Many of these bodies possess germicidal value, notably the oils of cinnamon and cloves. The oil of mustard is also a valuable disinfectant, but so irritating that the pure oil cannot be used. The use of powdered mustard in the autopsy-room will remove the foul odor from the hands more rapidly and completely than any other means.

Coal Oil or Petroleum.—While the disinfectant value of this substance is slight, its use in destroying the larvæ of insects, such as the mosquito, has given it an important position in preventing the spread of malaria and yellow fever. A small amount poured on a stagnant pool rapidly spreads over the surface and effectually destroys such larvæ.

Ferrous Sulphate (*Copperas*).—This salt has been much used, but possesses only feeble disinfectant powers. A 3 per cent. solution requires three days to kill the bacillus of typhoid fever. On account of its affinity for ammonia and sulphides it is an efficient deodorizer for temporary use, but cannot be relied upon to kill the bacteria producing the noxious gases.

Cupric Sulphate (Blue Vitriol).—This salt is quite an efficient disinfectant. In a solution of 1–3000 the spirillum of cholera is destroyed in ten minutes. A 5 per cent. solution will kill the typhoid bacillus in ten minutes. A solution of from 2 to 3 per cent. in strength can be relied upon to destroy all pathogenic organisms that do not form spores.

Zinc Sulphate.—This salt is a very feeble disinfectant. Pus cocci are not destroyed in two hours by a 20 per cent. solution. As a deodorizer it has about the same value and acts in the same way as ferrous sulphate.

Zinc Chloride.—A 2 per cent. solution will kill pus cocci after an exposure of two hours. It is therefore a much more powerful disinfectant than the sulphate.

Disinfection of Dejecta and Urine.—A 4 per cent. solution of calcic hypochlorite (chloride of lime) is most efficient and rapid for this purpose. A convenient solution contains 6 ounces of the salt to 1 gallon of water. The excreta should be received in a suitable vessel and immediately mixed with an equal bulk of the disinfectant. The contents of the vessel should be allowed to stand for one hour before emptying. A 20 per cent. milk of lime is just as efficient, and possesses the advantage of cleanliness and lack of odor. It should be used in the same quantity and allowed to act for the same length of time. A 5 per cent. solution of carbolic acid may be used, but should be allowed to act for at least four hours.

Disinfection of Sputum.—The chemical disinfection of tuberculous sputum is somewhat difficult on account of the large amount of albumin in it and the fatty matter associated with the bacillus of tuberculosis. Dilute solutions of bichloride of mercury are apt to be decomposed and rendered inert by the albumin. Carbolic acid is open to the same objection, but its combination with hydrochloric acid can be used successfully in a strength of 5 per cent. each. Milk of lime cannot be relied upon for this purpose. A 4 per cent. solution of calcic

hypochlorite (chloride of lime) is the best for general use, and the spit-cup should be kept nearly full of this solution. Sputum may also be disinfected by exposure to the action of steam in the steam sterilizer or by boiling for fifteen minutes. If napkins or old pieces of cloth are used for the reception of sputum, they may be immediately destroyed in a fire.

Disinfection after Postmortems.—After autopsies on infectious cases it is necessary to disinfect the table and fluid products coming from it prior to emptying into the sewer. The table may be successfully disinfected by a liberal sprinkling with 4 per cent. calcic hypochlorite solution. All fluids should be treated with an equal quantity of the same solution. The table should not be cleaned for at least one hour after application of the disinfectant. The same rule applies to the disinfection of the fluids—an exposure of at least one hour to the disinfectant before final disposition.

The Cadaver in Contagious Diseases.—In cases of death from a contagious disease all the orifices of the body should be packed with cotton soaked in a strong solution (1–500) of bichloride of mercury, the skin washed with a 1–1000 solution, and the cadaver wrapped in a sheet wet with the same. The funeral should be private and the body disposed of within twenty-four hours, preferably by cremation.

House Disinfection.—After infectious disease it is essential that the house or the apartment in which the patient has been confined should be disinfected. It is rarely necessary to carry out the process in more than two rooms; but should it be so, the process can be applied to the whole house.

After thorough bathing of the patient, preferably with an antiseptic soap, the individual should be wrapped in a clean sheet and removed to a clean room. All articles or materials that are of little value should be destroyed. All bedding, towels and the like should be placed in wooden tubs and covered with a 1–1000 solution of bichloride of mercury. The

room should then be made as nearly airtight as possible; this can be accomplished by pasting strips of paper over registers, cracks, spaces between window-sashes and the like. Formaldehyde gas is then passed through the keyhole into the room (or it may be generated by formaldehyde candles) in sufficient quantity to destroy the infectious element. The room should be sealed for at least twelve hours, after which time it may be opened and aired. The process is completed by washing all exposed surfaces in the room with 1–1000 bichloride of mercury. This latter requirement is not essential if the gaseous disinfection has been complete, but since we have no absolute knowledge on this point, the secondary washing should be carried out. This method can be considered reliable for surface disinfection, but for the interior of mattresses and stuffed furniture-cushions it is not certain. In all cases where absolute disinfection is demanded, such articles must be ripped apart and loosely exposed to the gas. They may be disposed of by fire or sterilized by steam under pressure. The latter method must necessarily be a matter of municipal control, and can be carried out only by means of suitable apparatus in the hands of a municipal disinfecting corps. Instead of formaldehyde, sulphur dioxide may be used for room disinfection, but in the light of present knowledge the formaldehyde method is superior.

CHAPTER IX.

THE PREPARATION OF INSTRUMENTS, LIGATURES, DRESSINGS, ETC., FOR SURGICAL PURPOSES.*

The purpose of this chapter is to explain the application of the principles set forth on the preceding pages to surgical technique. It has been shown that all objects about us may have bacteria on them, and that bacteria are present on all the surfaces of our bodies that come in contact with the air. All the care that is needed in working with bacteria in the laboratory, and more, must be exercised in surgical operations. Everything that has not been sterilized must be regarded as having the possibilities of infection in it. After the hands have been cleansed, if they touch the clothing or furniture, they must be cleansed again. If a sterilized instrument falls on the floor, it must be sterilized again. The same applies to dressings, sponges, ligatures or anything which is to be used about the wound.

The value of chemical germicides has probably been overrated in the past. They are used only to destroy the bacteria on living tissues and on articles that would be damaged by heat. They give less reliable results than boiling. Wherever boiling or steam sterilization is permissible, it should be used. With materials that may contain a small quantity of substance in which bacteria can grow, the fractional method of sterilization should be used (see page 51). With glass and metallic objects, obviously a single boiling can accomplish as much as boiling on three consecutive days.

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The failures in the practice of aseptic surgery are generally due to the hands of the operator and assistants and the skin of the patient.

The following formulæ have been selected from the many published, as they are successfully used by many surgeons, and meet the theoretical grounds of bacteriology as far as is possible with our present knowledge.

Sterilization of Hands.—There is no known method for perfect sterilization of the human skin. A close approach to sterility is reached by any one of the methods that has as its basis mechanical cleanliness.

Park's method: (1) Hands and forearms are thoroughly rubbed with a mixture of green soap and cornmeal, which serves to remove all the loose dirt and epithelium. Rinse carefully until hands and forearms are clean. (2) A paste of mustard flour and cold water is rubbed into the hands and forearms until they begin to sting. (3) Rinse in running sterile water; then soak in a hot 1–1000 bichloride of mercury solution for a few minutes, the fluid being well rubbed into the skin.

Fürbringer's method: (1) Thorough scrubbing of the hand and forearms with soft soap, water and a nail-brush for at least three minutes, especial attention being paid to the nails. (2) Removal of all fat and débris by rubbing hands and forearms while immersed in 95 per cent. alcohol. (3) Rinsing of hands and forearms in a 1–1000 bichloride of mercury solution, rubbing the fluid well into the skin.

Schatz's method: (1) Hands and forearms are cleansed by brisk scrubbing with soft soap and a clean brush for from three to five minutes. (2) Soaking in saturated solution of permanganate of potassium at a temperature of 110° F. until the hands and forearms are a deep mahogany brown. (3) Immersion in a saturated solution of oxalic acid, temperature of 110° F. until the skin is entirely decolorized. (4) Rinsing

with sterile lime-water to rid of excess of acid. (5) Washing in 1–1000 bichloride of mercury solution for one minute.

Weir's method: (1) Hands and forearms are scrubbed as in other methods. (2) A scant tablespoonful of chlorinated lime is moistened with enough warm water to make a thick paste. This is carefully rubbed into hands and forearms. (3) A piece of carbonate of soda one inch square and one-half inch thick is crushed and rubbed into the paste. From three to five minutes are thus employed. (4) Rinsing in sterile water and washing in a solution of $\frac{1}{5}$ of 1 per cent. of ammonia remove the odor of chlorine.

Rubber gloves are now quite widely employed. These can be readily sterilized and this obviates much of the tedious process required for sterilizing the hands.

E. R. McGuire* states that prolonged scrubbing with frequent changes of brushes in running sterile water will give the nearest approach to sterility. The use of antiseptics on the hands is not to be relied upon, for their precipitation by chemicals or normal tissue fluids may break up their combination with bacteria that were considered inactive or dead, but are not so in reality. He suggests the use of hot air by cabinet-bath or Kelly hot-air apparatus to "sweat" out of the glands in the skin as much as possible of their contents before the skin is cleansed.

Prolonged soaking of the skin in a soap poultice or strong antiseptic may damage and irritate the tissues, so that it is not advisable to prepare the field of operation more than twelve or twenty-four hours before the time set for an operation.

Maylard† recommends the sterilization of the skin by inunctions of oleate of mercury. The method employed is as follows: (1) Cleanse the skin in the usual way with soap and water. (2) Anoint freely and widely with hydrated lanolin-oleate of mercury, 20 per cent., and rub in; smear a piece of gauze with the same and leave until a second inunction

* *American Medicine.* February 28, 1903.
† *Annals of Surgery.* January, 1902.

is performed twelve hours later. Every case should be treated for at least twenty-four hours before operation; preferably forty-eight hours should be given, with at least two separate periods of "rubbing in" for about ten minutes on each occasion. (3) On the operating table the piece of gauze is removed, and the superfluous ointment rubbed off with a piece of sterile gauze.

To Prepare the Field of Operation.—Wash with green soap and water, scrubbing thoroughly and carefully, paying particular attention not to scrub hard enough to render the skin tender or to make abrasions. Shave parts with clean razor. Wash with ether and alcohol, to remove débris and epithelium, and cover with a sterile towel. If the skin of the patient is thick, a soap poultice may be left on, care being taken to see that the skin does not become macerated. After the patient is anesthetized the field is briskly scrubbed with sterile brushes, soap and water, washed with 1–1000 bichloride of mercury solution and covered with sterile towels.

It is important to remember that during an operation patient, operator and assistants may perspire and that in this way fresh masses of bacteria from the deeper parts of the glands may be brought to the surface of the skin. Careful attention must be paid to maintaining cleanliness during an operation. The patient's skin is kept covered with sterile towels, changed as often as they become soiled. For the surgeon's and assistants' hands *rubber gloves* do this perfectly. If an operator or assistant finds that the hands perspire during an operation, the use of rubber gloves becomes essential. Rubber gloves may be sterilized by boiling.

Instruments are best sterilized by contact with superheated steam, or steam under pressure for ten minutes, or by boiling in a 1 per cent. carbonate of soda solution. If soda is unavailable, use water that is actively boiling, as this avoids spotting and rusting of the instruments. Immediately after

use instruments should be thoroughly scrubbed with a brush and washed with soap and hot water and boiled, before being replaced in the instrument-case.

The practice of passing an instrument through a flame a few times cannot be relied on to destroy the bacteria that may be present.

Aspirating syringes, needles, trocars, drainage-tubes and glass nozzles are best sterilized by boiling for ten minutes. If syringes have leather washers (which should be avoided), they may be cleansed with hot water and soap, rinsed with alcohol, filled and refilled with boiling water ten or more successive times and placed in a 1–40 carbolic acid solution.

Instrument trays, ligature dishes, basins for sponges, etc., are to be sterilized by boiling for ten minutes, and protected from dust with sterile towels.

Catgut is made from the intestines of sheep, and sheep are subject to anthrax infection, while tetanus bacilli may occur in the intestine. Therefore, catgut must be sterilized by some method that will kill the spores of these organisms if present (see Bacilli of Anthrax and Tetanus, Part IV.). There are many methods devised for the preparation of sterile catgut that have as a basis an incorporation within the catgut of some antiseptic. They are open to the objection that any antiseptic introduced into the tissues acts as an irritant, aside from the fact that organisms may be liberated from partially absorbed catgut. This is seen in cases of late suppuration —ten to fifteen days after an operation.

Catgut comes in sizes from double zero up to No. 8. The sizes mostly used are 0 to 4. Catgut when ready for use should be smooth, soft, pliable and very strong; wiry catgut is apt to cut through tissues.

*Cumol method.** The catgut is rolled on glass spools, and

* Clark and Miller. *Bulletin Johns Hopkins Hospital.* Vol. XI. September, 1900.

these put in a glass beaker. The bottom of the beaker is covered with a layer of cotton on which the catgut rests. The beaker is heated with a Bunsen burner over a sandbath. The top of the beaker is covered with a piece of cardboard. Through a hole in the center of the cardboard a thermometer passes. Heat is now applied to the sandbath, and the temperature of the catgut slowly raised to 80° C. In this manner all moisture is driven out of the catgut. This degree of heat is maintained for one hour. Cumol* at a temperature of 100° C. is now added to the beaker, completely covering the catgut. The beaker should be covered with copper-wire netting to prevent ignition of the cumol, which is very inflammable. The temperature is then increased to 165° C., and kept at this point for one hour. The fluid is now poured off, and the catgut allowed to dry in the beaker on the sandbath at a temperature of 100° C. for two hours. It is then transferred to sterile jars or test-tubes until needed, or it may be preserved in sterile alcohol.

Formaldehyde catgut.† Three-quarter-inch glass spools are notched on each flange. The catgut is wound upon the spool tightly in one layer, and evenly, the ends passing over the flange of the spool in the notch; the longer end, after passing through the notch, goes through the barrel of the spool and is securely tied to the shorter end which has passed over the other notched flange. By thus winding the gut there will be enough for one or two ligatures or sutures of good length. Gut prepared by this process tends to contract forcibly, and on account of this strain must be held securely or it will shrink and be useless. The object of winding in a single layer, evenly, is to prevent overlapping or crossing of one strand over another. If in the process of soaking in formaldehyde and the

* Cumol is a fluid hydrocarbon, with a boiling-point somewhat above 165° C. It dissolves the fat in catgut. After boiling it has a brown color.

† Frederick. *American Journal Obstetrics.* Vol. LXXXIX. 1899.

consequent shrinking, one strand crosses another, the one next to the glass will be so pressed upon as to prevent hardening at that point. When the gut is boiled later in water, that point will gelatinize and break at the least strain. Formaldehyde comes in a 40 per cent. solution. We use a 3 per cent. solution, pouring one part of the 40 per cent. formaldehyde solution and thirteen parts of water into a wide-mouthed bottle. Immerse the spools in this solution for periods of time varying with the size of the gut. No. 0 is left in one hour. Nos. 1, 2 and 3 are given three, five and seven hours respectively. If left too long in the solution, the gut will become too hard, too brittle, and the strength will be impaired. Wash in running water for a longer time than it was in the formaldehyde solution. Up to this time the gut has not been sterilized. It has undergone a chemical change whereby it may be boiled without spoiling it. The sterilization of the gut consists in boiling for fifteen minutes, with the receptacles in which it is to be kept. With sterile forceps place the spools, each size by itself, in wide-mouthed ground-glass-stoppered bottles or in rubber-ringed fruit-jars, sterilized by boiling. Pour over the gut clean 95 per cent. alcohol with 8 to 10 per cent. of glycerin. To sterilize the glycerin it should be placed in a bottle in water and raised to the temperature of boiling water for half an hour.

To make *chromicized catgut* wind the spools as before. Place the spools in a solution of: bichromate of potassium, 1.5 grams; glycerin and carbolic acid, each, 10 c.c.; water, 1 liter. Allow them to remain in this solution for twenty-four hours. Take out and drain, allowing them to dry for a few hours. Then place in the formaldehyde solution and put through the same process as with formaldehyde catgut.

Kangaroo tendon, owing to its slow absorption, is used as a heavy retaining suture, and is prepared by washing the strands in ether to free from fat. Soak in a 4 per cent. solu-

tion of chromic acid for twenty-four hours. Then sterilize by the cumol method.

Silk may be sterilized by the fractional method (see p. 51) as this does not impair the strength as does boiling.

Silkworm gut is prepared by steam sterilization by the fractional method or by boiling in plain water for one-half hour. It should not be boiled in soda solution, as this spoils the gut.

Horsehair strands are cut into two-foot lengths, washed with soap and water and sterilized with steam by the fractional method. They make a very fine suture and are used where an inconspicuous scar is particularly desirable, as on the face. Only the finer grades are used for this purpose.

*Silver wire.** This material has the advantage over other suture materials of having a germicidal or at least a restraining influence on bacteria. If we remember that absolute sterilization of the skin is not possible by any means, we must see that in silver wire as a skin suture we have a safe and valuable material. Recent annealing by heating to a dull red increases the flexibility of the wire but almost totally destroys its germicidal property. This will reappear in a month and is not disturbed by boiling. Therefore prepare it by boiling for ten minutes in the 1 per cent. soda solution.

Sponges. The best absorbents to use in surgical work are those whose sterility is undoubted. Pads of gauze are easily sterilized by steam as for dressings. Sea sponges† may be prepared by beating with a wooden mallet to remove sand and dirt. Soak in a 1–64 solution of hydrochloric acid for twelve hours to remove lime deposits. Wash in running water. Soak for fifteen minutes in a saturated solution of permanganate of potassium, then place in a saturated solution of oxalic acid until they are perfectly bleached. After

* Bolton. *Transactions Association American Physicians.* 1894.

† McBurney. International Text-book of Surgery. June, 1900. P. 284.

immersion for half an hour in this solution rinse thoroughly in sterile water and put in a 1–1000 bichloride of mercury solution for twenty-four hours. Remove and place in 1–20 carbolic acid solution until required for use. At operation remove from solution, rinse out in normal salt solution, and place in receptacle filled with salt solution. If sea sponges are used on a septic case, they should be thrown away and no attempt made to resterilize them. If used on clean cases, they may be resterilized as above.

Dressings. The two materials universally used to dress wounds are "gauze" or cheesecloth and absorbent cotton. If they are properly sterilized, the impregnation of gauze or cotton with antiseptics does not add to their value. Gauze is usually cut in yard squares and folded compactly so as to make compresses. A number of compresses are wrapped with a piece of cotton cloth and the edges stitched loosely into a closed bundle. After sterilization by the fractional method, the bundles can be placed in sterile jars or receptacles and each bundle removed as needed. Ripping open the stitches gives untouched sterile bundles of compresses, convenient and handy for using. Cotton is sterilized by the fractional method in rolls or bundles as for gauze. These dressings should be warmed before being placed in the steam sterilizer or they will be unnecessarily wet when removed.

Irrigating solutions. Chemical germicides, such as bichloride of mercury when in solution, cause necrosis of tissue. Plain sterile water causes maceration of epithelium. Normal salt solution is the least irritating to the tissues and is the one most generally employed for irrigating purposes. It is 0.6 per cent. sodium chloride, prepared roughly by adding a teaspoonful of salt to the pint of water. This solution may be sterilized by boiling for half an hour on three consecutive days. It does not injure tissue, and may be freely used in operations for irrigating. It has no germicidal or antiseptic properties.

Accident wounds are generally lacerated or contused and may contain pathogenic bacteria. They should be promptly and carefully cleansed with sterile salt solution, wiped with sterile gauze and, if necessary, scrubbed vigorously with sterile soap and brush to remove all infectious dirt. When there is any doubt of this being accomplished it is better to dress such wounds wide open, filled with sterile gauze, for forty-eight hours or more. Retained blood-clots form a good medium for the development of bacteria so that drainage for a day or two is safer in doubtful cases.

Infected wounds. There is no known method for promptly sterilizing infected wounds without destroying tissue. An infected wound, if the infection be not too deep, may be sterilized by cauterizing with pure carbolic acid.

Care must be exercised in the application of antiseptic solutions in infected wounds for the antiseptic rarely penetrates as deeply into the tissues as the bacteria are found, therefore further necrosis of tissue and mechanical cleanliness are about all they accomplish.

After-treatment of wounds. Close attention to details is important in the technique of a first dressing after an operation. All instruments, irrigating fluids, bowls, basins, etc., are to be sterilized. When the dressing is removed the skin surrounding the wound should be cleansed by washing with salt solution or peroxide of hydrogen. The sutures or drainage should be removed with sterile forceps, and fresh sterile dressings applied. If a wound is found infected, all accumulations of blood-clot, pus, etc., should be gently and carefully washed out, and free drainage provided. In the treatment of infected wounds attention must be paid to maintaining mechanical cleanliness and avoiding infection with some organism which may not be already present.

It should be borne in mind that anything that tends to depress a patient's resisting powers, such as prolonged exposure to cold during an operation, loss of blood and infliction of a great degree of surgical shock, encourages infection.

PART III.

NON-PATHOGENIC BACTERIA.

The number of varieties of non-pathogenic bacteria is very large. Eisenberg* describes 376 species of bacteria, mostly non-pathogenic. Sternberg† enumerates 489 species, including the pathogenic varieties; but the majority, of course, are non-pathogenic. Flügge‡ considers about 500 species of bacteria. Migula§ recognizes nearly 1300, and Chester‖ about 800 species. Probably some of the bacteria which have been described as distinct species are in reality not different; but, on the other hand, it is also probable that a still larger number of species have not been described at all—how many, it is impossible to say. In a work of this character it is feasible to mention only a few of the commonest and best-known species of non-pathogenic bacteria.

Micrococcus agilis.—Found in water; coccus about 1 μ in diameter, usually appearing as diplococci, sometimes as streptococci and tetrads; liquefies gelatin slowly; grows at room temperature, on ordinary culture-media, forming a rose-red pigment on agar and potato. This micrococcus is remarkable in being actively motile; it possesses a flagellum. It is stained by Gram's method.

Micrococcus ureæ.—Found in decomposed, ammoniacal

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* *Bakteriologische Diagnostik.* 1891.
† Manual of Bacteriology. 1893.
‡ *Die Mikroörganismen.* 1896.
§ *System der Bakterien.* 1900.
‖ Manual of Determinative Bacteriology. 1901.

urine and in the air; coccus, 0.8 to 1 μ in diameter, occurring singly or in various combinations; does not liquefy gelatin; facultative anaërobic; grows rapidly, best at 30° to 33° C.; grows on ordinary gelatin, but best on special media; it decomposes urea, producing ammonia and carbon dioxide, which form ammonium carbonate.

Sarcinæ.—There is a large number of species of sarcinæ. They are common organisms in the air. They frequently contaminate plate-cultures. Many of the sarcinæ of the air present, in cultures, growths having brilliant colors, from which some of them are named; thus there are orange, yellow, rose-colored and white sarcinæ, and others.

Sarcina pulmonum.—Found in the air-passages of man; 1 to 1.5 μ in diameter, occurring in tetrads or cubes of eight cells; aërobic; does not liquefy gelatin; grows slowly, best at ordinary temperatures, preferably upon gelatin. It decomposes urine with the formation of ammonia. It is said to form endogenous spores which are extremely resistant to heat.

Sarcina ventriculi.—Found in the stomachs of man and of animals; 2.5 μ in diameter, occurring in cubes of eight cells or more; it does not liquefy gelatin; aërobic; grows on ordinary culture-media; the growths tend to become yellow. Small numbers of sarcinæ may occur in the normal human stomach; the presence of large numbers indicates the existence of abnormal fermentative processes.

Bacillus fluorescens liquefaciens.—Found in water and putrid fluids; very common; appears as a small rod, actively motile; aërobic, but somewhat variably; liquefies gelatin; grows rapidly at ordinary temperatures upon the usual culture-media. It forms a pigment having a beautiful greenish-yellow fluorescence, best seen in transparent media; the growth on potato has a brown color. Does not stain by Gram's method and does not form spores.

Bacillus fluorescens putidus.—Found in water; a short

rod with rounded ends; actively motile; does not liquefy gelatin; aërobic; does not form spores; grows rapidly at the ordinary temperatures upon the common media. Gelatin cultures give off a powerful, foul odor of trimethylamin. It produces a greenish, fluorescent pigment, best seen in transparent media; on potato the growths form a thin, gray to brown, slimy layer.

There are several other fluorescing bacilli, mostly found in water.

Bacillus Indicus.—Found by Koch in the stomach contents of an ape in India; a fine short bacillus with rounded ends; motile; does not form spores; facultative anaërobic; liquefies gelatin; grows rapidly, best at 35° C. upon the ordinary media; produces a brick-red pigment. Very large doses injected into rabbits caused death in three to twenty-four hours.

Bacillus prodigiosus.—Widely disseminated in the atmosphere of certain places; a short bacillus with rounded ends, in form often nearly like the micrococci; facultative anaërobic; not motile, as a rule; does not form spores; liquefies gelatin rapidly; grows rapidly, best at 25° C. on the ordinary culture-media; milk is coagulated; gas forms in sugar-media; cultures on potatoes give off a foul odor of trimethylamin. A brilliant red color, which develops only in the presence of oxygen, appears in cultures. The pigment appears as granules outside of the bacteria.

Bacillus violaceus (of Berlin).—Found in water; a slim rod with rounded ends which may form threads; actively motile; facultative anaërobic; liquefies gelatin rapidly; forms endogenous spores placed near the centers of the bacilli; grows rapidly, and not at high temperatures, upon ordinary media, forming a deep, violet-colored pigment. There are several bacilli related to this one.

Bacillus amylobacter (Clostridium butyricum; Bacillus butyricus, Prazmowski).—Found widely distributed in nature

in decomposing vegetable material and in the stomachs of ruminant animals; a large, thick rod with round ends, often arranged in chains; actively motile; anaërobic; forms spores which are located in the center of the bacillus and give it a spindle-shaped form, or at one end, when it has the outline of a tadpole; has not been cultivated satisfactorily on ordinary media; grows best at 35° to 40° C.; decomposes carbohydrates with the formation of butyric acid; decomposes cellulose. Organisms of similar form have been found as fossils belonging to the carboniferous period.

Bacillus butyricus (Hueppe).—Found in milk; appears as a small, irregular rod, also forming threads; very actively motile; aërobic; rapidly liquefies gelatin; forms centrally located spores; grows best at 35° to 40° C.; grows rapidly on ordinary media; coagulates milk, redissolving the coagulum, producing also butyric acid. A large number of bacteria, both aërobic and anaërobic, produce butyric acid fermentation.

Bacillus megaterium.—Obtained by de Bary from cooked cabbage-leaves; common on plants and earth; a large bacillus with rounded ends, often forming chains; motile; slowly liquefies gelatin; aërobic; forms spores, especially in potato cultures; grows rapidly at room temperature on the ordinary media.

Bacillus mesentericus vulgatus (Potato bacillus).—Found on potatoes; common in earth; a large, long rod with rounded ends, often forming long chains; motile; it is stained by Gram's method; liquefies gelatin; aërobic; forms spores; grows rapidly, best at about 20° C.; grows on ordinary media, forming on potato a thin, wrinkled membrane which spreads rapidly over the surface. It coagulates milk, redissolving the coagulum. It possesses numerous flagella. The spores are extremely resistant to heat.

Bacillus phosphorescens Indicus.—Obtained from seawater; a small, thick, rod-shaped bacillus with rounded ends,

also forming threads; actively motile; not stained by Gram's method; liquefies gelatin; aërobic. It grows slowly, best between 20° and 30° C., upon the usual media, except milk and potato. Its cultures, when old, especially when on animal nutrient-media and in the presence of certain sodium salts, are phosphorescent in the dark.

There are various other bacilli which produce phosphorescence, some of which do not liquefy gelatin.

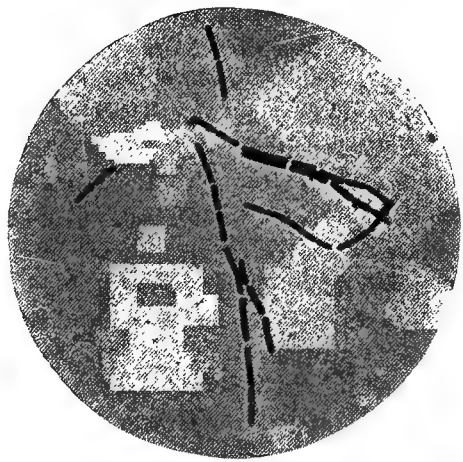

FIG. 54.—BACILLUS SUBTILIS. (× 1000.)

Bacillus mycoides (Bacillus ramosus; *Wurzelbacillus*).—Found in the earth and in water; very common; a large, short bacillus with rounded ends, often forming chains and threads; slightly motile; liquefies gelatin; aërobic; forms centrally located, oval spores; grows rapidly at room and incubator temperatures upon the usual media. It is said rapidly to decompose albumin with the formation of ammonia.

Bacillus subtilis (Hay bacillus).—Found on hay, in the air, water, ground and decomposing fluids; very common;

a large bacillus somewhat resembling the anthrax bacillus in form, with rounded ends, often forming chains or long filaments; motile; possessing flagella; liquefies gelatin; aërobic; it is stained by Gram's method. It may have large, centrally located spores, which form best on potato at about 30° C. The spores are extremely resistant to heat and to chemical germicides. It grows best at about 30° C. upon the ordinary culture-media; milk is peptonized. Bacillus subtilis may easily be isolated in pure culture by adding finely cut hay to tubes of bouillon; placing these in the steam sterilizer for five or ten minutes; then letting the tubes develop in the incubator. Plates made from the bouillon will probably show colonies of the Bacillus subtilis only, as the steam may be expected to have destroyed all organisms except its very resistant spores. The hay bacillus is entirely without pathogenic properties.

Bacillus erythrosporus.—Found in decomposing fluids and water; a slim bacillus with rounded ends; motile; does not liquefy gelatin; facultative anaërobic; forms oval, red-colored spores, two to eight in each filament; grows rapidly, only at ordinary temperatures; produces a greenish-yellow, fluorescent pigment. On potato it forms a limited, reddish growth, becoming nut-brown.

Bacillus cyanogenus (Bacterium syncyanum; Bacillus lactis cyanogenus; Bacillus of blue milk).—A bacillus of variable size, with rounded ends; motile; spore formation doubtful; is aërobic; not stained by Gram's method; grows rapidly at ordinary but not so well at incubator temperatures on the usual culture-media; does not liquefy gelatin; produces a grayish-blue pigment, brighter in acid media, at ordinary temperatures; milk is not coagulated or rendered acid.

Bacillus acidi lactici (Hueppe).—Found in sour milk; a short, plump rod; not motile; does not liquefy gelatin; facultative anaërobic; grows on the ordinary media; in milk causes development of lactic acid with precipitation of casein and

production of gas and alcohol. It belongs in the same group as B. coli communis and B. lactis aërogenes (see Part IV.).

There are numerous other bacteria, such as the Bacterium acidi lactici, which cause the formation of lactic acid in milk.

Bacterium ureæ.—A short, thick bacillus with rounded ends; not motile; aërobic; found in ammoniacal urine; grows slowly at room temperature upon gelatin, which is not liquefied; decomposes urea; forms ammonium carbonate.

Bacterium Zopfii.—Found in the intestines of hens, in water and in fecal matter; a bacillus 0.75 to 1 μ broad and 2 to 5 μ long; may form threads. Actively motile; does not liquefy gelatin; aërobic; involution forms are often seen and they have been described as spores; grows rapidly, best at 20° C. upon gelatin; forms branching zoöglœæ. It is a member of the same group as B. proteus (see Part IV.).

Spirillum rubrum.—Found by Esmarch in the putrefying cadaver of a mouse; short spirals twice the breadth of the cholera spirillum, usually with one to three turns; in bouillon growing into long spirals; motile, with flagella; spore formation doubtful; facultative anaërobic; does not liquefy gelatin; grows slowly, best at about 37° C. on the ordinary media; produces a wine-red pigment only when the air is excluded.

Spirillum or Spirochæta dentium.—Found in the mouths of healthy persons, on the margins of the gums when they are covered with a dirty deposit; long spirals with several windings, uneven in thickness; has not been cultivated.

Spirillum sputigenum.—Found in the human mouth in healthy persons at the margin of the gums; curved rods or short spirals which resemble the spirillum of cholera in form; has not been cultivated.

Spirillum rugula (Vibrio rugula).—Found in swamp water, in fecal matter and in the tartar of the teeth; a curved rod 0.5 to 2.5 μ broad and 6 to 8 μ long, having one flat spiral wind-

ing; motile, with flagella at the ends; probably anaërobic; forms spores located at the ends.

Spirillum volutans.—Found in swamp water; very long spirals with several turns; 1.5 to 2 μ broad and 25 to 30 μ long; motile, with a flagellum at each extremity. The protoplasm is granular.

Spirillum undula.—Found in putrefying infusions containing organic matter; a rather short spiral form with three

FIG. 55.—SPIRILLA FROM SWAMP WATER. (× about 500.)

turns or less, about 1 μ thick and 8 to 12 μ long; actively motile, with a tuft of flagella at each extremity; has been cultivated on agar.

Spirillum or Spirochæta plicatile.—Found in swamp water; spiral forms of various lengths; sometimes 100 to 200 μ long; actively motile.

The spirilla (vibrios or comma-shaped forms) closely resembling the spirillum of cholera, will be considered in connection with that organism. A form of chronic pseudomem-

branous inflammation of the pharynx has been attributed to an organism called the fusiform bacillus or spirillum of Vincent.*

Higher Bacteria.—Certain organisms (beggiatoa, thiothrix, leptothrix, cladothrix, actinomyces or streptothrix) of more complicated structure than most bacteria, but resembling them in many respects, are called "higher bacteria." They consist of definite filaments which are usually made up of rod-

FIG. 56.—SPIRILLA FROM SWAMP WATER SHOWING FLAGELLA (LÖFFLER STAIN). (× 1000.)

shaped elements, but the relation between these elements is very intimate. Some of them (beggiatoa, thiothrix) contain sulphur granules. Many of them occur in water. There are forms among them which are found attached to some object by one end of the filament (thiothrix). Some of them (actinomyces or streptothrix) have branching filaments, which are rarely seen among the lower bacteria (see page 108). Often

* Mayer. *American Journal Medical Sciences.* Vol. CXXIII. 1902. P. 187.

one end of the filament becomes specialized for the purposes of reproduction. The fungus of actinomycosis is the best known of this group. There are many other members, however, both pathogenic and non-pathogenic. Most of them require still further study. The tubercle bacillus and other acid-proof bacilli which resemble it have some points of resemblance with actinomyces (see B. tuberculosis, Part IV.).

Leptothrix buccalis.—Found in the mouth cavity. This name has been applied to large, twisted, thread-like organisms, in which segments can be demonstrated with difficulty or not at all. Apparently, different organisms have been described under this name. Vignal claims to have cultivated a Leptothrix buccalis. Miller recognizes two principal species, neither of which could be cultivated,—*Leptothrix innominata*, which shows no transverse divisions, and which is stained faintly yellow by iodine; and *Bacillus buccalis maximus*, in which the transverse divisions are distinct, and which is stained brownish-violet by iodine. Miller's *Leptothrix maxima buccalis* is similar to the last except in lacking the iodine reaction.

A variety of leptothrix, or a nearly related organism, appears to be the most frequent cause of the form of gangrenous inflammation of the mouth and genitals called noma. It stains faintly by Gram's method. It does not grow on ordinary media.* Another organism of this group has been described which is pathogenic to a number of domestic animals.†

Yeasts and Moulds.—In the course of bacteriological work one constantly encounters yeasts and moulds, which, although not bacteria, must nevertheless be understood and recognized to avoid error. Accidental contamination of tubes or plates is likely to be the result of the growth of some of these forms.

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* Blumer and MacFarlane. *American Journal Medical Sciences.* November, 1901.

† It has also been called "necrosis bacillus," and "Streptothrix cuniculi." Pearce. *University of Pennsylvania Medical Bulletin.* November, 1902.

The yeasts generally go by the name of *saccharomyces*, of which there are several species. The *Saccharomyces cerevisiæ* is the ordinary yeast of alcoholic fermentation. Some of the yeasts present colored growths—red, white and black. They consist of large, oval cells, which readily stain with the aniline dyes. They multiply by the protrusion of a little bud from the cell, which develops into a new cell. In an actively germ-

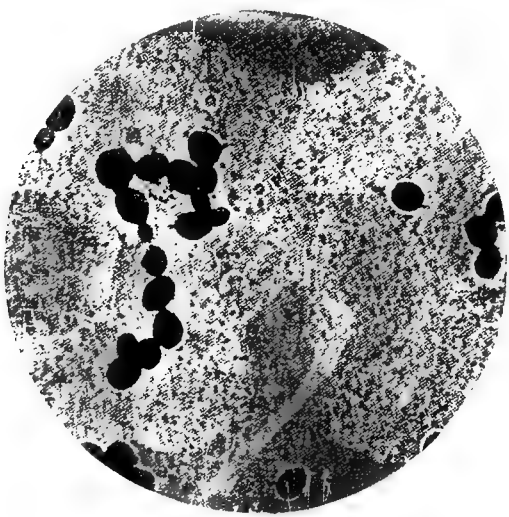

FIG. 57.—YEAST CELLS STAINED WITH FUCHSIN. (× 1000.)

inating growth of yeast these budding cells are readily distinguished (Fig. 57).

Yeasts have been found that were pathogenic to animals. They have also been supposed to be the cause of some malignant tumors, but this view has been, for the most part, abandoned.

Among the moulds the varieties most commonly encountered are the *mucor*, the *penicillium*, the *aspergillus* and the *oïdium*. There are various species of each of them. They

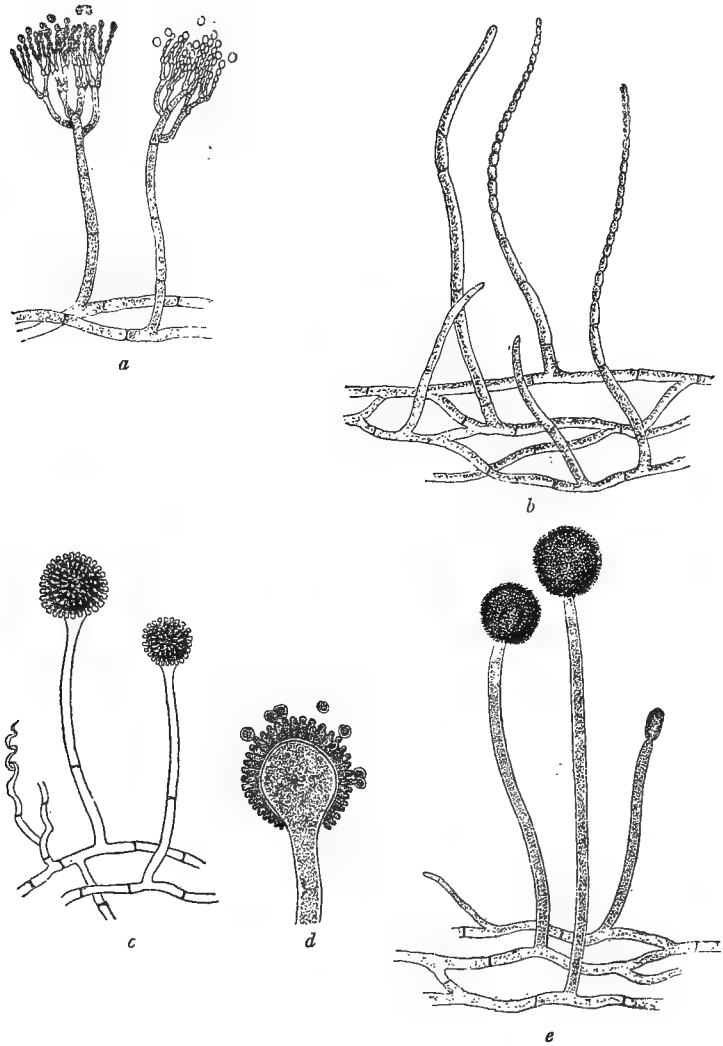

FIG. 58.—(*Baumgarten.*)

a. Penicillium glaucum. *b*. Oïdium lactis. *c*. Aspergillus glaucus. *d*. The same more highly magnified. *e*. Mucor mucedo.

consist of cells arranged end to end, making a thread-like body called a *hypha*. The threads are matted together and form a *mycelium*. Certain threads project upward from the mycelium, and on them are borne *spores*. The arrangement of the spores is characteristic in each variety of mould (Fig. 58). A group of organisms exist which have affinities both with yeasts and mould-fungi. Some of them are pathogenic. The form of infection of the mouth called thrush is due to a fungus of this class, which is generally considered an oïdium. A chronic inflammatory affection of the skin (blastomycetic dermatitis) is due to related organisms.* The sporotricha of Schenck † which produces chronic subcutaneous abscesses, may be mentioned here, provisionally. A number of skin affections, such as tinea favosa and tinea trichophytina, are due to fungi, which have some similarity to those above mentioned.

Among the mould fungi, several species of aspergillus and of mucor are pathogenic. Man, as well as the lower animals, may be affected. In man the lungs may be involved in a broncho-pneumonia (pneumonomycosis), usually due to aspergillus, and often secondary to some preëxisting disease of the lung. Mould fungi, especially aspergillus, may grow in the external ear (otomycosis). The growth is usually superficial. These fungi rarely produce lesions in other organs.

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* Ricketts. *Journal of Medical Research.* Vol. VI. 1901. Hyde and Montgomery. *Journal American Medical Association.* June 7, 1902.

† Hektoen. *Journal Experimental Medicine.* Vol. V.

PART IV.

PATHOGENIC BACTERIA.

Suppuration and Allied Conditions.—The occurrence of suppuration is characterized by certain appearances which we are accustomed to describe under the name of inflammation. The study of inflammation belongs to pathology, and cannot be considered fully here. However, certain evidences which are characteristic of the suppurative variety of inflammation need to be outlined on account of their relation to the action of the pyogenic bacteria.

In a suppurating area, as is well known, the blood-vessels are dilated, and the lymph-spaces become filled with serum. Leukocytes are attracted to the neighborhood in large numbers, by positive chemotaxis, and crowd the small veins and capillaries. The leukocytes, by reason of their ameboid movement, pass through the walls of the vessels at little openings filled with cement-substance, situated between the lining endothelial cells. According to the theory of phagocytosis, they are bent on finding the irritant which has led to the inflammation, and upon attacking it and rendering it harmless. At the point which appears to be the center of the inflammatory area there is usually, but not always, a necrosis of the cells of the tissue; this constitutes the central slough or the familiar core found in some boils. The necrosis is to be attributed to poisons formed by the micrococci. In sections cut through such an abscess the nuclei of the necrotic cells in the center fail to take the nuclear stain; the necrotic mass does not stain,

or takes the dye diffusely and irregularly, and it is broken up into fine granules.

The cells of the tissues surrounding the necrotic area are mingled with large numbers of polynuclear leukocytes, which enclose the area of irritation.

The nuclei of the cells near the center of the abscess are frequently broken up into a number of small fragments, which indicates the commencement of their destruction. In sections through small abscesses it is possible, by means of a double stain of carmine, followed by Gram's method, to bring out the histological character of the tissue, and at the same time to stain the common pyogenic bacteria, which are usually found near the center of the abscess in large numbers, even making masses visible with a low power of the microscope. It is often possible by this method to demonstrate masses of micrococci filling up the lumina of capillaries in which they are lodged as emboli.

The production of pus in the center of the abscess is due to the liquefaction of the necrotic tissue, which apparently results from the action of some peptonizing ferment. In the liquid thus formed immense numbers of the polynuclear leukocytes are found floating, and they constitute the greater part of the so-called *pus-cells*. The nuclei of these cells are obscured by clouds of extremely fine granules. The granules are of an albuminoid nature, and are dissolved by acetic acid, when the nuclei become visible. The nuclei generally consist of three, four, five or more portions. The presence of the fine albuminoid granules in the pus-cells is to be counted as a degenerative change. Although it is possible to produce suppuration experimentally by the introduction of sterilized irritants, such as croton oil, into the tissues of animals, in all cases met with in practice suppuration is due to the action of pyogenic bacteria.

Specimens of pus will nearly always be found to contain

bacteria, which can be demonstrated by cultivation, and, as a rule, also in smears made and stained upon cover-glasses. The bacteria are generally found outside the pus-cells. In the case of the gonococcus and the Diplococcus intracellularis meningitidis they are characteristically found in pairs, inside of, or at least attached to, the pus-cells. The character of the suppuration differs somewhat with the different species of pyogenic bacteria. The kind of abscess above described—localized and having a central slough, usually rather slow in progress—is typical for the Staphylococcus pyogenes aureus, which is prone to produce circumscribed areas of suppuration. The Streptococcus pyogenes, on the other hand, oftener leads to suppuration of a more diffused character, such as we see in cellulitis and erysipelas; but either organism may, at times, produce the effects usually characteristic of the other. Pus having a blue or green tinge generally owes the color to the presence of the Bacillus pyocyaneus. The commonest pus-producing organism is then the Staphylococcus pyogenes aureus, and next to that the Streptococcus pyogenes. Among the other pyogenic bacteria the following may be named:

Staphylococcus pyogenes albus, including Staphylococcus epidermidis albus; streptococcus of erysipelas (probably identical with Streptococcus pyogenes); gonococcus; Diplococcus intracellularis meningitidis; Staphylococcus pyogenes citreus; Micrococcus tetragenus; Micrococcus pyogenes tenuis, which may be the same as the Micrococcus lanceolatus; Staphylococcus cereus albus and flavus.

Pus-formation may also be due to Micrococcus lanceolatus, Bacillus pyocyaneus, Bacillus proteus, Bacillus coli communis, Bacillus pyogenes fœtidus, Bacillus pneumoniæ (of Friedländer), Bacillus aërogenes capsulatus, the ray fungus of actinomycosis, and possibly the bacillus of bubonic plague. Besides these organisms, there are others whose effects are usually more marked in a specific way which sometimes form pus, as the

bacilli of diphtheria, tuberculosis, glanders and typhoid fever. Frequently two or more species of pyogenic bacteria will be found associated.

The table below, quoted from Dowd, shows the frequency of the occurrence of various pyogenic bacteria in 135 cases of different types of suppuration.

	Cellulitis, 51 Cases.	Infected Fresh Wounds, 17 Cases.	Old Granulating Wounds, 18 Cases.	Healing Wounds: Stitches, 5 Cases.	Furuncles, 7 Cases.	Abscesses, 37 Cases.
Streptococcus pyogenes alone	9	3	..	..	..	..
Streptococcus pyogenes predominant	23	3	..	..	..	8
Streptococcus pyogenes relatively few	3	1	6	..	..	1
Staphylococcus pyogenes aureus alone	11	1	1	1	7	6
Staphylococcus pyogenes aureus predominant	8	2	..	..	..	1
Staphylococcus pyogenes aureus relatively few	13	3	..	..	..	2
Staphylococcus pyogenes or epidermidis albus alone	1	4	2	4	..	2
Staphylococcus pyogenes or epidermidis albus predominant	..	1	..	..	..	..
Staphylococcus pyogenes or epidermidis albus relatively few	10	5	3	..	..	6
Staphylococcus cereus albus	3	1	2	..	..	1
Staphylococcus citreus	1	..	2	..	..	1
No growths on agar	..	..	..	..	..	11
Very few growths on agar	..	..	3	..	..	3
Bacillus pyocyaneus	..	..	1	..	..	3
Bacillus coli communis	..	..	..	..	..	3
Overgrown	4	..	2	..	..	1
Few undetermined colonies	12	2	5	..	..	5

The condition of the animal's tissues is of great importance in determining whether or not suppuration will occur when exposed to infection. It will be seen that we are repeatedly subjected to infection with pyogenic bacteria, but that in most cases suppuration nevertheless does not occur. The local conditions have an important influence in determining infection.

Regions of hyperemia, edema, anemia or necrosis are especially liable to suppuration, as are tissues which have been bruised, lacerated, strangulated or otherwise damaged. Furthermore, the general condition of the patient is of great importance. Chronic diseases and conditions of exhaustion or depression dispose to suppuration, and the depraved condition of the tissues in diabetes renders the sufferer from this disease especially liable to it. These facts have already been enumerated in a previous chapter (page 155). In the lower animals we find that it is often very difficult to produce suppuration artificially with the ordinary pyogenic bacteria. In rabbits the subcutaneous introduction of Staphylococcus pyogenes aureus frequently fails to produce an abscess. Suppuration is likely to result, however, if an irritant body like a piece of sterilized potato or sterilized glass be introduced along with the bacteria.

There are probably a number of other specific predisposing causes in the animal body about which we are only beginning to obtain an understanding. The weakening of the alexins, the absence of opsonins and other intricate conditions are probably subject to great variability, and may serve to explain the tendency to infection at certain times.

Pyogenic bacteria are most frequently introduced into the body through the agency of injuries and wounds of various sorts. They are very widely disseminated in nature and have been found clinging to various objects, especially in city houses. The infection of a wound with pyogenic cocci, when the suppuration is of a spreading character, such as is most characteristic of streptococcus infection, is known in everyday language as "blood-poisoning." It is possible for infection to take place around hair-follicles through the unbroken skin. In such instances the suppurative inflammation first shows itself in a minute red pimple with a hair in the center. The pimple presently becomes a pustule. The process may cease at this point,

or it may be only the commencement of a large carbuncle with a central slough. Such infection has been produced experimentally on the human skin by rubbing in cultures of Staphylococcus pyogenes aureus. It is, furthermore, the constant experience of post-mortem examiners that infection may occur around the hair-follicles when no wound of the skin has been inflicted.

In many instances, infection with the pyogenic bacteria follows upon some preëxisting infection; this happens, for instance, in tuberculosis, when tuberculous lungs become infected with Streptococcus pyogenes, leading to the formation of a cavity. It is a common occurrence in gonorrhea, after the acute stage of the disease has passed, when we find the gonococcus in the pus, mingled with other pyogenic micrococci. Secondary infection with pyogenic bacteria is frequently due to the Streptococcus pyogenes, often also to the Micrococcus lanceolatus.

Sometimes it is impossible to detect the point of entrance of pyogenic organisms. In view of the observation that tubercle bacilli pass through the uninjured mucosa without leaving any local lesion, but setting up the disease in places remote from the point of entrance, it may be surmised that the pyogenic organisms may enter the body without leaving any trace of their point of entry.

The severe general symptoms, familiar to every physician, often accompanying acute suppuration, indicate the formation of toxic bacterial products and their absorption. Experimental evidence of the formation of such toxic products is not so clear, however, for the pyogenic organisms as for some of the other bacteria. It has been shown that cultures of Staphylococcus pyogenes aureus, in which the bacteria have been killed, are capable of producing suppuration in the lower animals.

The pyogenic bacteria play a somewhat different part in

producing disease, which is fully as important as the typical suppuration seen in an abscess. This happens when the suppurative condition is complicated by other pathological processes, or when there is inflammation of another variety without suppuration at all; or they may produce lesions which are not inflammatory in a strict sense. These differences in their action depend largely upon the organ affected. One such condition is osteomyelitis, which is suppuration occurring in bone, but which does not prevent the ordinary picture of pus-formation owing to the hard and unyielding character of the tissue. Other conditions of very great importance are meningitis, pericarditis, pleuritis, croupous and broncho-pneumonia, peritonitis and endocarditis. It will be observed that these affections are, for the most part, inflammations of the serous membranes. Such inflammations, when they are produced by pyogenic bacteria, are likely to be of great severity, accompanied by the formation of fibrinous exudates; pus-formation may or may not be present. We find that the cause at times is the Staphylococcus pyogenes aureus; this is often the case in malignant endocarditis. Generally speaking, in such inflammations the Streptococcus pyogenes, the Staphylococcus pyogenes aureus and the pneumococcus occur most commonly, although they are by no means the only organisms found. Many cases of peritonitis show the presence of B. coli communis, either in combination with other bacteria or alone.* This is explained by the proximity of the intestine, and especially by the frequent occurrence of peritonitis after perforation of the intestine.

In inflammations of mucous membranes the common pyogenic organisms are usually the cause, though other organisms are occasionally responsible. In acute bronchitis, pneumo-

* Flexner. Etiology, etc., of Peritonitis. *Philadelphia Medical Journal.* November 12, 1898.

cocci and streptococci were found by Ritchie* to be the commonest causes.

In inflammations of the middle ear the principal causes are the pneumococcus, the streptococcus and the Staphylococcus aureus and albus.†

In 25 cases of acute cystitis in woman Brown‡ found B. coli communis, 15 times; S. pyogenes albus, 5 times; S. pyogenes aureus, twice; B. typhosus, once; B. pyocyaneus, once; B. proteus vulgaris, once.

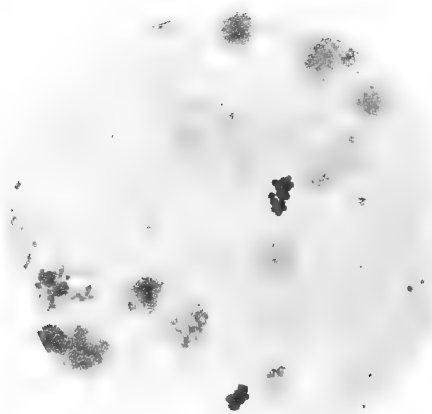

FIG. 59.—STAPHYLOCOCCUS PYOGENES AUREUS IN PUS STAINED BY GRAM'S METHOD. (× 1000.)

A number of investigators have recovered organisms resembling the pyogenic cocci from cases of acute articular rheumatism. Most frequently a diplococcus or short streptococcus has been found, which has sometimes produced arthritis and endocarditis when inoculated into rabbits. But

* Ritchie. *Journal Pathology and Bacteriology*. Vol. VII. December, 1900.

† Hasslauer. *Centralblatt für Bakteriologie*. XXXII. Ref. 1902, p. 174. Compare *ibid.*, pp. 240 and 246.

‡ *Johns Hopkins Hospital Reports*. Vol. X. 1902.

Cole* has shown that this is not the specific organism for acute articular rheumatism.

From a point where there is suppuration or other localized infection, pyogenic bacteria may enter the circulation and become widely disseminated throughout the body. That happens very commonly in malignant endocarditis. In this manner secondary or metastatic abscesses may be produced in the most diverse organs.

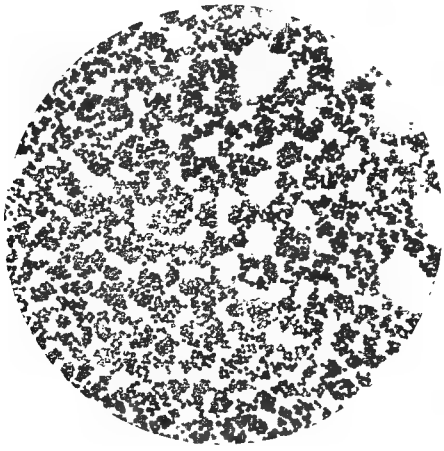

FIG. 60.—STAPHYLOCOCCUS PYOGENES AUREUS, PURE CULTURE. (× 1000.)

The term *pyemia* is used to describe the dissemination of pyogenic bacteria in the circulating blood, with the formation of metastatic abscesses.

Staphylococcus pyogenes aureus.—A micrococcus of variable size, arranged in irregular clumps, sometimes in pairs; about 0.8 to 0.9 μ in diameter; not motile (Fig. 60). It stains

* Cole. Experimental Streptococcus Arthritis in Relation to the Etiology of Acute Articular Rheumatism. *Journal of Infectious Diseases.* Vol. I. No. 4. Nov. 5, 1904. P. 714.

by Gram's method; it is a facultative anaërobe; grows rapidly, best at 30° to 37° C. It liquefies gelatin. Upon gelatin plates small colonies appear at the end of about two days. It grows well upon all the culture-media. Milk is coagulated. It does not lead to fermentation with the production of gas, but produces various acids.

The growths in the first place are pale, subsequently becoming golden-yellow in color, but only in the presence of oxygen. This color appears well on all media, and is especially distinct on potato. Sometimes the color is slow in developing.

In a fresh, moist condition the organism is killed by ten minutes' exposure to 58° C.; in a desiccated condition it requires a temperature of 90° to 100° C. to destroy it. It is not killed by drying. In the same specimen the micrococci may have quite different resisting powers to chemical germicides. Some of the individual cells are destroyed by 1–1000 solution of bichloride of mercury in five minutes; others survive exposure to this solution for from ten to thirty minutes. (Abbott.)

Sterilized cultures introduced into animals may produce local suppuration. The cells contain intracellular toxic substances and otoxins.*

As has already been mentioned, the Staphylococcus pyogenes aureus is the commonest of the pyogenic bacteria in man. It has been obtained from a great variety of sources, and appears to be able to exist as a saprophyte. It has been found on the skin, in the mouth, in the nasal and pharyngeal mucus, and also in the alimentary canal. It has furthermore been detected in the air and in dust. It appears to find the conditions necessary for its existence in the vicinity of human habitations.

Cultures of the Staphylococcus pyogenes aureus vary considerably in virulence. These variations are sometimes to be explained through cultivation on unfavorable media or repeated

* Morse. *Journal Experimental Medicine.* Vol. I., p. 613.

transplantation from one medium to another; but at times the diminished virulence is due to unknown causes. The lower animals used for experiments are not as readily infected as man. The local introduction in rabbits or guinea-pigs of a part of a culture of Staphylococcus pyogenes aureus may be entirely without effect. The use of a very large dose, or the addition at the same time of some kind of irritant, may produce an abscess. Large amounts of cultures in bouillon may often be injected into the peritoneal cavity of the dog without effect, when the simultaneous addition of a piece of sterile potato or an injury to the gut may lead to fatal peritonitis. Introduction of fluid cultures into the venous circulation of the rabbit generally produces metastatic abscesses in the kidneys, the heart-muscle and the voluntary muscles, and causes death.

FIG. 61.—STAPHYLOCOCCUS PYOGENES AUREUS. GELATIN CULTURE, ONE WEEK OLD.

In man this organism produces suppuration of a localized character, such as we are familiar with in boils and carbuncles. It has been shown to be the usual cause of infectious osteomyelitis. Osteomyelitis has been produced experimentally in rabbits by the injection of the Staphylococcus pyogenes aureus, both with and without previous injury to the bone of the animal. Ulcerative endocarditis has on numerous occasions been shown to be due to this organism. It has been found possible to produce ulcerative endocarditis experimentally in animals by the injection of the Staphylococcus pyogenes aureus when the valves of the heart have first been mechanically injured. The Staphylococcus pyogenes aureus has also been

found in acute abscesses of the lymph-nodes, tonsils, parotid gland and mammary gland, in suppurating joint affections and empyema. It appears, furthermore, in acute inflammation of the serous membranes,—pleuritis, pericarditis, peritonitis,—although less frequently than the Streptococcus pyogenes.

Staphylococcus pyogenes albus.—In form and manner of growth this organism behaves like the Staphylococcus pyogenes aureus, with the exception that it produces no colored growths and its cultures appear white. Its pathogenic properties are less marked, and it is a less frequent cause of suppuration than the Staphylococcus pyogenes aureus. It has, however, been found in acute abscesses on numerous occasions.

Staphylococcus epidermidis albus.—According to Welch, the epidermis of man contains with great regularity the organism to which he gave the above name, and which he considers to be a variety of Staphylococcus pyogenes albus. It grows, liquefies gelatin, and coagulates milk more slowly than the ordinary Staphylococcus pyogenes albus. It is, furthermore, possessed of less marked pus-producing tendencies. Welch found it impossible to sterilize the skin so as to remove this micrococcus from it. The organism is usually innocuous. It has been found in healthy wounds on numerous occasions. It is capable of causing trouble in wounds when necrotic or strangulated tissues are present, or where a foreign body like a drainage-tube has been left in the wound. It is a common cause of stitch abscesses.

Streptococcus pyogenes.—Appears as micrococci arranged in chains, usually in pairs, when the adjacent cocci may be flattened. Sometimes the chains are very long. The diameters of the cocci vary from 0.4 to 1 μ. Attempts have been made to create varieties of streptococci according to the length of the chains. On that basis a Streptococcus brevis and a Streptococcus longus have been described.

The Streptococcus pyogenes is not motile. It stains by Gram's method. By the method of Hiss (page 46) capsules may sometimes be demonstrated. It is facultatively anaërobic; grows best in the incubator; more slowly at room temperature, and does not liquefy gelatin. In gelatin plates it produces small, round, white, punctiform colonies which are slow of development, and are visible only after about three days. It grows on the ordinary media, but according to some authors

FIG. 62.—STREPTOCOCCUS PYOGENES, FROM A PURE CULTURE. (× 1000.)

it does not grow on potato. Milk may or may not be coagulated. The growths are never very luxuriant, and may die out after a few transplantations.

It is killed by exposure to 52° to 54° C. in ten minutes. The Streptococcus pyogenes occurs frequently on the mucous surfaces of the healthy body. It is often found in pus, especially pus of spreading inflammations of the kind known as cellulitis. This organism is the commonest infectious agent in puerperal fever, metritis and peritonitis. It occurs com-

monly in inflammations of the serous membranes—pleuritis, pericarditis and peritonitis. It has been discovered many times in ulcerative endocarditis and in bronchopneumonia. It is frequently present in the false membrane found in genuine diphtheria. It is also the cause of many of the pseudomembranous or so-called "diphtheritic" affections of the throat where the Klebs-Löffler bacillus of diphtheria is wanting. These cases may be indistinguishable clinically from genuine

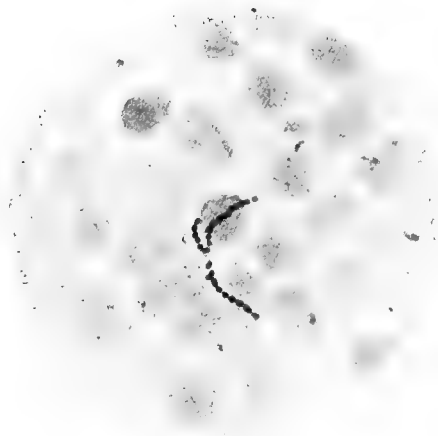

FIG. 63.—STREPTOCOCCUS PYOGENES IN PUS, GRAM'S STAIN. (× 1000.)

diphtheria, and their nature can be revealed only by bacteriological examination. They are, however, as a rule, milder than genuine diphtheria. The pseudomembranous affections of the throat which occur in scarlet fever and measles are generally caused by the Streptococcus pyogenes, although those diseases may be complicated by genuine diphtheria. Streptococci are very commonly present in the throat in scarlet fever,* and sometimes occur in the blood. Some observers believe that scarlet

* Weaver. *American Medicine.* April 18, 1903.

fever is caused by streptococci. Streptococci are very often found in the pustules of small-pox, and may also appear in the blood.

The Streptococcus pyogenes is pathogenic for mice and rabbits, but the virulence is very variable. That may sometimes be increased by passing through a number of animals in succession, but is rapidly lost in artificial cultures. It is said that the virulence is best maintained when cultures on gelatin, after forty-eight hours' growth, are kept in a cool place, as in the ice-chest. Marmorek has shown that the virulence may be maintained or increased by growing it first in a mixture of human blood-serum (or that of the ass or the horse) with bouillon and then inoculating it into the body of a rabbit, alternating these procedures. In this way it is possible to obtain cultures of very high virulence. A serum of uncertain value derived from an immunized horse or ass has been prepared by Marmorek for the cure of streptococcus infection.

FIG. 64. — STREPTOCOCCUS PYOGENES, CULTURE ON AGAR (SLIGHTLY ENLARGED).

A number of other sera have been prepared for combating streptococcus infection. These have been used in cases of streptococcus infection in human beings; also in cases of scarlet fever. The results appear somewhat encouraging, although still uncertain.

It is said that streptococci may be agglutinated by serum from animals immunized with streptococcus.

Coley has recommended a bouillon culture of Streptococcus pyogenes (or of erysipelas), in which the Bacillus prodigiosus was afterward grown, to be administered by injection, after sterilization of the cultures by heat, in cases of inoperable sarcomatous tumors. These injections appear in some cases to have accomplished remarkable and wholly inexplicable cures.

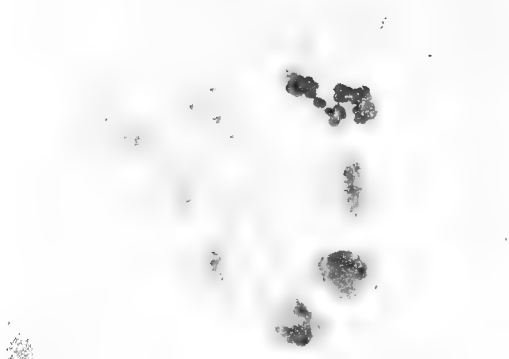

FIG. 65.—MICROCOCCUS TETRAGENUS IN PUS FROM A LARGE ABSCESS ON THE ARM, SHOWING CAPSULE, GRAM'S STAIN AND EOSIN. (× 1000.)

Streptococcus of Erysipelas.—The cause of erysipelas is a streptococcus which in all essential respects—in its morphology, its growth on culture-media, its behavior with stains and its pathogenic properties—corresponds to the Streptococcus pyogenes. It is probable that these organisms are identical.

Micrococcus tetragenus.—Found in the cavities in the lungs of pulmonary tuberculosis, in sputum and in pus. The micrococci are enclosed in a transparent capsule, best seen in preparations from the tissues of inoculated animals, and are

arranged in pairs or in fours; about 1 μ in diameter; not motile; stain by Gram's method. It grows well at the room temperature, but rather slowly; is a facultative anaërobe; does not liquefy gelatin. Gelatin plates show little, white, punctiform colonies, which, with the low power, are finely granular, and have a peculiar glassy shimmer; in stab-cultures the growths appear as little colonies along the line of puncture. On agar, round white colonies form, which do not tend to spread. It produces a thick, slimy film on potato and a broad, white, moist growth on blood-serum. This organism is only occasionally found in pus. It is pathogenic for white mice and guinea-pigs, but not for gray mice and rabbits. It may produce septicemia or only a localized suppuration in guinea-pigs. In white mice general septicemia results on inoculation and the Micrococcus tetragenus is found in the blood and in the great viscera. White mice usually die in from two to six days; guinea-pigs in from four to eight days from inoculations.

Micrococcus lanceolatus (Micrococcus pneumoniæ crouposæ; Micrococcus Pasteuri; Diplococcus pneumoniæ; Micrococcus of Sputum Septicemia; Streptococcus lanceolatus Pasteuri; and Pneumococcus of Fränkel).—This organism was discovered by Sternberg in his saliva in 1880, and afterward demonstrated to be the cause of lobar pneumonia by Fränkel and Weichselbaum. The micrococci usually occur in pairs. The pair of micrococci, in its most typical form, appears like a couple of curved triangles with their bases close to each other. The outline is usually described as being lancet-shaped. The micrococci are frequently oval or round; they often form chains. In preparations made from the blood of infected animals or from pneumonic sputum each pair of micrococci is seen in stained preparations to be surrounded by a capsule; the capsule is not usually seen in preparations made from cultures. For methods of demonstrating the capsule see pages 45 and 46. The pneumococcus is not motile. It stains by Gram's method,

which also is useful in demonstrating the capsule. It is facultatively anaërobic. It grows only at elevated temperatures, preferably about 35° to 37° C. Gelatin is not liquefied. It grows well upon agar, upon blood-serum and upon Guarnieri's medium (p. 69). It does not grow upon potato. Milk usually becomes acid, and may or may not be coagulated. The colonies are seen in their characteristic form upon agar, and are developed after about forty-eight hours, appearing as minute, whitish, translucent, circular growths.

FIG. 66.—PNEUMOCOCCUS OF FRÄNKEL IN SPUTUM OF PNEUMONIA, GRAM'S STAIN AND EOSIN. (× 1000.)

It is killed by an exposure to 52° C. for ten minutes.

It is best cultivated from the blood of an animal which has been infected with the sputum of a case of lobar pneumonia. Cultures need to be transplanted every few days; they cannot usually be propagated more than a month or two months.

The virulence of the organism for animals diminishes rapidly in cultures. It frequently grows as a streptococcus on

artificial media. When virulent, it is pathogenic for mice and rabbits; less so for guinea-pigs. In these animals it is likely to lead to fatal septicemia in twenty-four to forty-eight hours when introduced subcutaneously or into the peritoneum or when liquid cultures are injected intravenously. The blood often contains great numbers of the diplococci. The virulence of the organism is very variable. In the sputum of a case of lobar pneumonia, early in the disease, it is likely to be virulent. The virulence is best maintained by repeated inoculations into mice or rabbits.

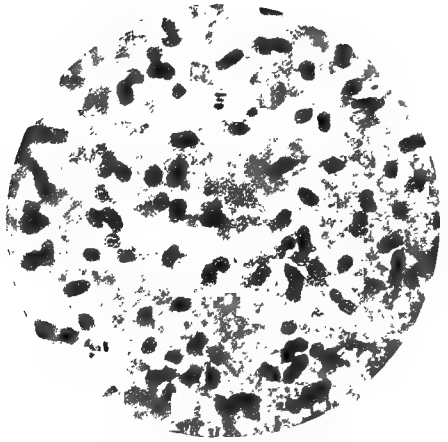

FIG. 67.—PNEUMOCOCCUS, SHOWING CAPSULE, FROM PLEURITIC FLUID OF INFECTED RABBIT, STAINED BY SECOND METHOD OF HISS. (× 1000.)

The Micrococcus lanceolatus has been detected very frequently in the mouths of healthy individuals. But under these conditions it is not, however, pathogenic for animals in many instances, being found virulent in only from 15 to 20 per cent. of such cases. It is the specific cause of croupous or lobar pneumonia in man. In that disease the characteristic lesion consists of an inflammation of the lung, involving large areas—usually one or several lobes. An exudate is poured into the

air-vesicles, which in the early part of the disease contains red blood-cells, imparting the rusty color to the sputum. The principal element in the exudate is fibrin. The formation of fibrin produces the liver-like consolidation or "hepatization." The diplococci can readily be demonstrated by the Gram method in sections of pneumonic lung, which are best stained by carmine and gentian-violet.

The Micrococcus lanceolatus can be detected in large numbers, sometimes almost unmixed with other bacteria, in the rusty sputum of lobar pneumonia, often showing the peculiar unstained capsule. On account of its liability to be mixed with other forms of bacteria its presence in the sputum of cases suspected of being pneumonia is not of very great value in differential diagnosis, especially since it is so commonly present in the normal mouth. In a suspicious case its appearance in sputum in nearly pure culture may be significant.

Cultures from the blood of cases of pneumonia, where a large amount of blood is taken, have shown the presence of the pneumococcus in a considerable proportion of the cases, especially when severe or fatal.

The Micrococcus lanceolatus is often also the cause of bronchopneumonia and of meningitis. It produces inflammations in other situations as well, the most important being pleuritis, pericarditis, endocarditis and arthritis. The Micrococcus lanceolatus may produce pseudomembranous inflammation* and also ordinary suppuration, although not very commonly.

G. and F. Klemperer claim to have obtained toxins from cultures of the pneumococcus, and to have established immunity in animals with the development in the blood of antitoxic substances. Similar attempts have been made by Wash-

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* Cary and Lyon. *American Journal Medical Sciences.* Vol. CXXII. 1901.

bourn and others, but the interpretation of their results at the present time is not clear. The agglutination reaction has been claimed to occur with the pneumococcus, but it does not yet appear to have any practical value in diagnosis.

Organisms related to the pneumococcus have been described under the names of pseudopneumococcus* and Streptococcus mucosus.†

The organism named by Rosenbach *Micrococcus pyogenes tenuis* is probably only a variety of the pneumococcus.

Micrococcus melitensis.—A micrococcus found by Bruce in cases of Malta fever. It is a round or slightly oval organism, about 0.5 μ in diameter, occurring singly, in pairs or in short chains. It is usually said to be non-motile, though flagella have been described. It is stained by ordinary aniline dyes, but not by Gram's method. It grows slowly, even in the incubator, and more slowly at ordinary temperatures. In gelatin the growth is feeble; there is no liquefaction. On agar pearly white growths appear after three or four days. Bouillon becomes turbid, and later a sediment is formed. On potato there may be slight invisible growth.

Malta fever occurs chiefly about the Mediterranean. It has been observed in India, in the Philippine Islands and in Porto Rico.

It is a chronic febrile disease, but not very fatal, accompanied by pains in the joints and perspiration. At autopsies the organisms may best be recovered from the enlarged spleen. Accidental infection in man has occurred from pure cultures on a number of occasions. The disease may be reproduced in monkeys by inoculation with pure cultures. The agglutination reaction is positive in this disease. The diagnosis is best made by applying this test to the blood-serum of the

* Richardson. *Journal Boston Society of Medical Sciences.* Vol. V. 1901.
† Howard. *Journal Medical Research.* Vol. VI. 1901.

patient, with a known pure culture of Micrococcus melitensis.* For this purpose a suspension of an agar culture is made in normal salt solution. The diluted serum is added so as to secure a dilution of about 1–50, but the dilutions used have varied widely. Precipitation quickly follows agglutination. According to Craig, the test may be made on a slide, examining with the microscope as for the typhoid bacillus (see Serum-test for Typhoid Fever).

Diplococcus intracellularis meningitidis.†—Found in the exudate of cerebro-spinal meningitis by Weichselbaum; a micrococcus about the size of the common pyogenic cocci; grows in fours, but more often in pairs consisting of two hemispheres separated by an interval which does not stain; usually found within the pus-cells, in which respect it resembles the gonococcus. It is stained by ordinary methods with the aniline dyes, and is decolorized by Gram's method. It does not grow at the room temperature, but only in the incubator; gelatin is not available as a culture-medium. There is no growth on potato and scanty growth on agar or in bouillon. The development is most abundant upon Löffler's blood-serum, when round, white, shining, viscid-looking colonies with sharp outlines may be seen in twenty-four hours. The serum is not liquefied. Upon agar, or better upon glycerin-agar, the colonies are flat, round, translucent, viscid-looking, having a yellowish-brown color under the low power. The organism should be transplanted to fresh media frequently, as it rapidly loses its power of reproduction. Many of the tubes inoculated with the original material or with pure cultures show no growth.

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* Musser and Sailer. *Philadelphia Medical Journal.* December 31, 1898, July 8, 1899. Strong and Musgrove. *Ibid.* November 24, 1900. Curry. *Journal Medical Research.* Vol. VI. 1901.

† The writer is indebted for the brief statement which it is possible to give here chiefly to the exhaustive Report to the Massachusetts Board of Health by Councilman, Mallory and Wright, 1898. The photograph was made from a preparation kindly furnished by Dr. Mallory.

It is moderately pathogenic for guinea-pigs and rabbits when inoculated into the pleura or peritoneum. Meningitis and encephalitis have been produced in the dog and goat by inoculation in the meninges.

This organism appears to be the principal if not the only cause of epidemic cerebro-spinal meningitis. The lesion consists of a purulent inflammation of the pia and arachnoid, extending into the brain-substance, over the cord and along the nerves. General invasion of the tissues of the body seems not to occur, but focal areas of pneumonia may be present.

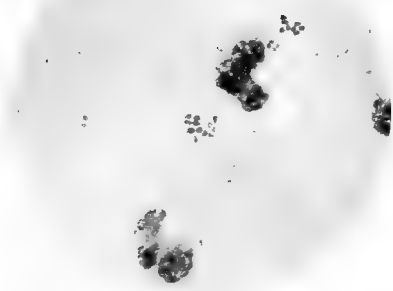

FIG. 68.—DIPLOCOCCUS INTRACELLULARIS MENINGITIDIS AND PUS-CELLS. (× 1000.)

Spinal puncture in the lumbar region is recommended as a means of diagnosis. The puncture should be made early, and the fluid should be examined with the microscope and by cultures.

Micrococcus gonorrhœæ (Gonococcus of Neisser).—Found in pus in cases of gonorrhea. The micrococci generally are in pairs, occasionally in groups of four. The cocci are flattened, the flattened sides facing each other, and they are often compared to a pair of biscuits. The long diameter of the pair of biscuit-shaped elements is about 1.25 μ. The organisms are

usually found attached to the epithelial cells or inside of the pus-cells; they are also found in smaller numbers floating free in the fluid. They stain with ordinary aniline dyes, for example, Löffler's methylene-blue, but not by Gram's method.

The fact (1) that the cocci always occur inside of the pus-cells, (2) that they are in pairs of biscuit-shaped micrococci, (3) that they are not stained by Gram's method, will serve to distinguish the gonococcus from all the other ordinary

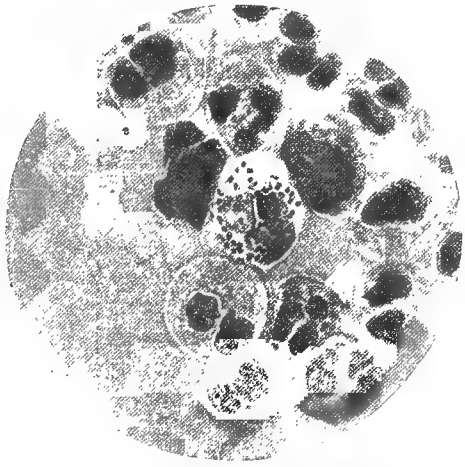

FIG. 69.—GONOCOCCI AND PUS-CELLS. (× 1000.)

pus-forming bacteria. There are other diplococci (pseudo-gonococci), probably non-pathogenic, which have been found occasionally in the vulvo-vaginal tract and in the urethra, which, it is said, are also decolorized by Gram's method. Such organisms are not likely to present all the points mentioned as characteristic of the gonococcus. The recognition of the gonococcus in the discharges of a case of acute gonorrhea is usually an easy matter. It must be admitted, however, that in cases having chronic discharges, when its detection is most to be

desired, the diagnosis may become very difficult and is frequently impossible, except by culture-methods, owing to secondary infection with the ordinary pus-forming or other bacteria, which may be present in larger numbers than the gonococci themselves.

The gonococcus grows only in the incubator, and cannot therefore be cultivated upon gelatin. Its cultivation is, in fact, a matter of some difficulty. The medium usually selected is a mixture of agar with human blood-serum. The blood-serum from the placental blood or pleuritic or peritoneal transudates, or hydrocele fluid, has been employed. Human urine, sterilized by filtration through porcelain, added to the mixture of blood-serum and agar, improves its character, according to some writers. A convenient medium is one consisting of one part of human serum derived from a pleuritic effusion, added to two parts of a 2 per cent. nutrient agar previously sterilized. The two are mixed in tubes while fluid and cooled while in an inclined position, and sterilized between 65° and 70° C. by the fractional method on six consecutive days. They are afterward tested in the incubator for two days.

The colonies of the gonococcus are very small, grayish-white, circular, translucent; appearing after from twenty-four to forty-eight hours. They may attain a diameter of 1 to 2 mm. The gonococcus will occasionally develop on ordinary glycerin-agar or Löffler's blood-serum medium, but the growth is likely to be feeble and cannot be relied on. The cultures live for a considerable time if kept from drying. The gonococcus is not known to produce urethritis or conjunctivitis in any of the lower animals. In the peritoneum it may cause suppurative inflammation in mice and guinea-pigs. Reproduction of the disease in man has been effected by experimental inoculation with pure cultures. Besides being the cause of gonorrheal urethritis and infection of the cervix uteri, the gonococcus has been isolated from cases of vaginitis in little girls and from

gonorrheal conjunctivitis. It has been found to be the cause of many cases of pyosalpinx, as well as of gonorrheal proctitis, arthritis, myocarditis and endocarditis; these conditions complicating gonorrhea may also be secondary or mixed infections.

Bacillus of Soft Chancre (of Ducrey).—A small, oval bacillus, usually occurring in chains. It stains with ordinary aniline dyes, but not by Gram's method. It has been cultivated on human blood-agar (also rabbit blood-agar; the medium deteriorates in a few weeks—Davis). It is cultivated with difficulty. It is found in the pus of soft chancre or chancroid, usually mixed with other organisms. It has been demonstrated in sections of the ulcers. There seems to be uncertainty with respect to its occurrence in buboes. Ducrey was able to secure it in pure culture by successive inoculations on the human skin. Although this bacillus has not yet been sufficiently studied, there seems little doubt that it is the cause of soft chancre.*

Bacillus pneumoniæ (of Friedländer), or *Bacillus mucosus capsulatus*.†—A short bacillus with rounded ends, sometimes growing out to a greater length; sometimes occurring in pairs; surrounded by a capsule which is seen only in preparations made from the tissues of infected animals, and is not seen in cultures. This bacillus is not motile. It does not form spores. It stains with the ordinary aniline dyes, but does not stain by Gram's method. It is aërobic and facultatively anaërobic. It may be cultivated at ordinary temperatures, but grows better in the incubator. It does not liquefy gelatin. Stick-cultures in gelatin develop a round, flat knob at the point where the puncture enters the surface of the gelatin, making what is called a "nail-shaped" growth; the growth in gelatin is white; in old

* Davis. *Journal Medical Research.* Vol. IX. 1903.

† Howard. *Philadelphia Medical Journal.* February 19, 1898. Curry, Howard, Perkins. *Journal Experimental Medicine.* Vols. IV. and V.

cultures the various media acquire a brown color. Dextrose and lactose are fermented by it; in cultures on potato, gas is formed, causing a frothy appearance; milk is not coagulated. It does not produce indol.

The thermal death-point is about 56° C. It is pathogenic for mice, less so for guinea-pigs and rabbits. This bacillus is sometimes found in the healthy mouth and nose. It has been known to cause inflammation, especially in the vicinity of the mouth, nose and ear, bronchopneumonia, and more rarely empyema and meningitis. It was described by Friedländer as the specific cause of lobar pneumonia; but more recent investigations indicate that it is comparatively seldom found in pneumonia.

There are various capsulated bacilli (capsule bacilli of R. Pfeiffer and others) which closely resemble the bacillus of Friedländer, and at least belong to the same group. The bacillus of ozena, which has often been found in that disease is very similar. B. lactis aërogenes and B. coli communis also have many points in common with the Friedländer bacillus.

Bacillus of Rhinoscleroma.—A short bacillus with rounded ends, often united in pairs, also growing to a greater length; surrounded by a capsule; not motile; stained by the ordinary aniline dyes. It is much like the bacillus of Friedländer, but some writers state that it retains Gram's stain more tenaciously than that organism; this may be doubted, however. The organism has been cultivated. It is a facultative anaërobe. It grows rapidly, best in the incubator. It does not liquefy gelatin; its growth in gelatin stick-cultures resembles that of the bacillus of Friedländer. It grows on the ordinary media. Gas may be developed upon potato.

It is pathogenic for mice and guinea-pigs, less so for rabbits. Its virulence is less than that of Friedländer's bacillus.

It has been obtained from the tissues of cases of rhinoscleroma. Rhinoscleroma is a disease characterized by a chronic tuber-

cular thickening and swelling of the skin around the nose and similar swelling of the nasal mucous membrane, sometimes followed by ulceration. It is commonest in Austria and Italy. It has been seen in America only very rarely.

The organisms may be stained in the diseased tissues, but their detection is a matter of considerable difficulty, and they are not always found. It is not yet certain that they are the cause of rhinoscleroma.

Bacillus mycogenes.*—A plump, short bacillus, less than 1 μ in breadth, possessing no flagella, non-motile, does not form spores; capsules are seen in preparations from tissues of inoculated animals and in milk cultures, rarely in preparations from agar cultures. The organism occurs singly or in pairs, and even in longer filaments. Gram positive in tissues, but negative in cultures.

The growth on agar is porcelain white and viscid. In all liquid media viscidity is very marked. Gelatin is not liquefied. Coagulated blood-serum not liquefied. "Nail-head" growth shows in stab cultures. Milk is coagulated in one to five days. Casein not digested. Litmus is reduced. Growth on potato is brown and slimy, but there is no gas formation. Indol negative. None of the sugars are fermented.

Very pathogenic for rabbits and guinea-pigs. Rabbits are killed in eighteen hours by subcutaneous injection of $\frac{1}{100}$ c.c. of a twenty-four hour beef-broth culture, guinea-pigs in less than fifteen hours by the same dose.

Bacillus pyocyaneus.—A slim bacillus with rounded ends. It is motile. It does not form spores. At 56° C. it is killed in ten minutes. It is decolorized by Gram's method. It is aërobic; grows well at ordinary temperatures; liquefies gelatin, and grows on the ordinary culture-media. Cultures present

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*Ralph T. Edwards. *Journal of Infectious Diseases.* Vol. II. No. 3. 1905.

a blue or green color, especially in transparent media. This color is not confined to the growth itself, but a blue or green fluorescence spreads over the whole medium. In an old agar culture the color may become very dark. The pigment forms in the presence of oxygen, and is due, at least in part, to the ptomaine, pyocyanin. On potato the growth is usually brown; the surrounding medium may be tinged with green. Milk is coagulated and peptonized and an acid reaction is developed.

FIG. 70.—BACILLUS PYOCYANEUS, PURE CULTURE. (× 1000.)

Indol is formed in Dunham's peptone solution. Coagulated blood-serum is liquefied.

The Bacillus pyocyaneus seems to be rather widely distributed in nature; it has been found on the skin, in normal feces, also in diarrheal discharges and in dysentery. It is the cause of the color in blue or green pus. It has frequently been demonstrated in pus, but oftenest perhaps, in mixed infections. It has been found in various abscesses, in otitis media, peritonitis, appendicitis and bronchopneu-

monia. It has been known to produce general septicemia.* It is pathogenic for guinea-pigs and rabbits, in whom it may produce septicemia. In animals it may lead only to local suppuration, from which they may recover, being made immune from subsequent infection with this organism. It appears that an antagonism exists between the products of the Bacillus pyocyaneus and the anthrax bacillus. Rabbits which have been inoculated with cultures of the anthrax bacillus may recover if they are injected shortly after with a culture of the Bacillus pyocyaneus.

There appears to be a whole group of fluorescent organisms of slightly different characters which closely resemble one another, all classed as pyocyaneus.

Bacillus proteus.—A bacillus with rounded ends, varying much in length, breadth 0.4 to 0.6 μ; frequently appearing as short ovals like micrococci; sometimes growing out into long filaments, so that it is said to be pleomorphic. Rounded involution forms occur. It is not stained by Gram's method. It is motile. Spore formation has not been observed. It is aërobic and facultatively anaërobic. It grows rapidly at ordinary temperatures. This organism was originally described by Hauser as three different species—*Proteus vulgaris*, which was said to liquefy gelatin rapidly, *Proteus mirabilis*, which liquefied gelatin slowly, and *Proteus Zenkeri*, which did not liquefy gelatin. It seems probable that these organisms were, in fact, varieties of the same species, now called Bacillus proteus. Upon gelatin-plates the colonies present a characteristic phenomenon, when seen under the low power, in the projection of processes which subsequently change their form and position, and which may become entirely detached from the original

* Lartigau. *Philadelphia Medical Journal.* September 17, 1898. *Journal Experimental Medicine.* Vol. III. 1898. Perkins. *Journal Medical Research.* Vol. VI. 1901.

colony, so that the surface of the gelatin may become covered with so-called "swarming islands."

The proteus grows on the usual media, tending to produce a foul odor, decomposition and alkaline reaction. In urine it converts urea into ammonium carbonate.

This organism is one of those which were formerly described under the name of Bacterium termo. It is among the most common and widely distributed bacteria. It has been found in decomposing animal and vegetable substances, in the feces,

FIG. 71.—BACILLUS OF BUBONIC PLAGUE.—(*Yersin.*)

in the urine in cystitis and in the discharges of children suffering from cholera infantum. It appears that this organism may occasionally be pathogenic to man, causing pus formation, peritonitis and even general infection.* Cultures injected in considerable amounts may be pathogenic to animals.

Bacillus of Bubonic Plague.—An oval or short rod-shaped bacillus, with rounded ends, sometimes possessing a capsule. It is not motile. It does not form spores. With the aniline dyes the ends stain more deeply than the middle; this is called polar

* Ware. *Annals of Surgery.* Vol. XXXVI. 1902.

staining; by Gram's method it is decolorized. It is aërobic. It grows at ordinary temperatures, but better in the incubator. It grows on most media. The growths are grayish-white. Gelatin and blood-serum are not liquefied. In bouillon, the medium remains clear, while a granular deposit forms on the sides and bottom of the tube. In bouillon to which a few minute drops of sterile oil, as cocoanut oil, have been added, a growth takes place from the under side of the oil drops. Such growths extend down, and are called stalactite growths. The stalactites break off, with the slightest disturbance.

Remarkable involution forms appear on agar containing 3 per cent. of common salt. The stalactite growths and the forms occurring on salt-agar are considered the most characteristic cultural peculiarities.*

It is sometimes sensitive to drying, but may sometimes survive prolonged drying. When spread in thin layers, it is killed in three to four hours by direct sunlight; in a few minutes by steam at 100° C., and in one hour by one per cent. carbolic acid.† It is pathogenic for rats, mice, guinea-pigs, rabbits and a number of other animals.

In man it appears usually to enter through wounds of the skin. Other possible avenues of infection are the air-passages, the mouth and the gastro-intestinal tract. In plague three different clinical forms are to be recognized—the bubonic, the pneumonic and the septicemic. The *bubonic* form is commonest. The point in the skin at which the inoculation takes place seems generally to exhibit no inflammatory reaction. The lymph-nodes are generally swollen, especially the deep inguinal and axillary nodes. The swollen lymph-nodes may suppurate. The suppurating nodes are often infected simul-

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* Wilson. *Journal Medical Research.* Vol. VI. 1901.

† See Rosenau. Viability of Bacillus pestis. Marine Hospital Service. Hygienic Laboratory Bulletin. No. 4. 1901.

taneously with micrococci. The bacilli are numerous in the enlarged lymph-nodes, but may be detected in the other organs of the body and in the blood. The organism is formed in the fluid drawn with a hypodermic needle from bubo during life. It may be cultivated from this fluid, and recovered from rats and guinea-pigs inoculated with it. In the *pneumonic* or pulmonary form the bacilli occur in the sputum, and may be tested in the same manner. This type of the disease is said to be very fatal. In the *septicemic* form no primary bubo is found; but a bubonic case may become septicemic, and this form is very fatal.

During epidemics of plague it has been noted that rats may die in large numbers, and plague bacilli have often been recovered from the bodies of such rats. The systematic destruction by health departments of all the rats possible is important where an epidemic is present or is feared. The same applies to mice. The agency of fleas as carriers of the bacilli has been suggested, but has not yet been proved; this is equally true as to flies.

The greatest care must be used in working with the bacillus of plague. A number of fatal results have occurred through it in laboratory investigators.

Haffkine has devised a method of protective inoculation against plague consisting of the injection of cultures of plague bacilli which have been sterilized by heat, with a little carbolic acid added. An active immunity which is quite lasting, it is maintained, may be secured by this method in some days. The injection is sometimes followed by considerable constitutional disturbance. This method seems likely to be of considerable value.

Yersin and others have prepared protective sera on the same general principles used in making other sera for effecting passive immunity. The results so far obtained with these sera are very encouraging.

An agglutination reaction has been described; but this is not likely to be of great value in diagnosis.

The period of incubation in this disease is from two to seven days. It has occasionally appeared in civilized countries during recent times, though not to a very serious extent. Among the localities of importance to us it has recently visited the Philippine Islands, California and Mexico. It has ravaged

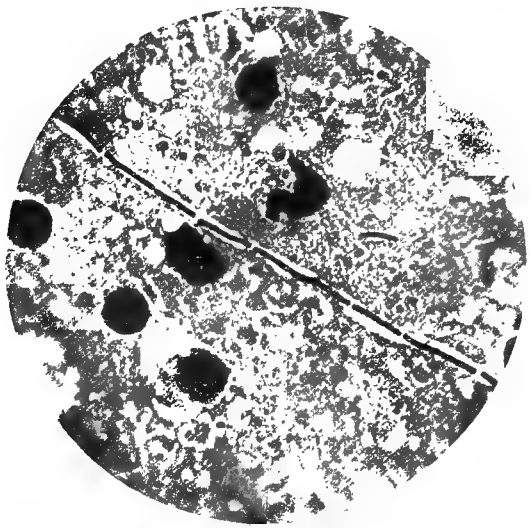

FIG. 72.—BACILLUS AËROGENES CAPSULATUS, SMEAR PREPARATION FROM RABBIT'S LIVER. (× 1000.)

the southeastern part of Asia within a few years. In the Middle Ages, and in succeeding centuries, it devastated many of the countries of Europe, where it was one of the most important of the pestilences that went in those days by the name of the "plague." It appears to have been the disease known in English history as the "black death."*

* For further details concerning plague consult articles by Barker, Novy and Flexner. *Transactions of the Association American Physicians.* 1902. Calvert. *American Medicine.* January 24, 1903.

Bacillus aërogenes capsulatus.—A thick bacillus, 3 to 6 μ in length, frequently capsulated, discovered by Welch and Nuttall. The capsules may be found in preparations from animal tissues, but rarely in cultures. It sometimes forms spores, chiefly in cultures on blood-serum. The vegetative forms are destroyed at 58° C. moist heat in ten minutes, but the spores withstand boiling nearly eight minutes. It is not motile. It stains by Gram's method. It is anaërobic, and is readily cultivated by Buchner's method for anaërobes. It grows best at the body temperature, but will grow at the room temperature. It may liquefy gelatin slowly or not at all. The growths are whitish. In media containing lactose, dextrose or saccharose it produces an abundance of gas; but according to Welch, it is also able to form gas from proteids. Milk is coagulated, and the reaction becomes acid. Gas forms upon potato, where the growth is thin and grayish-white.

It occurs in the intestine of man and various other animals, in soil, sewage and water. It is

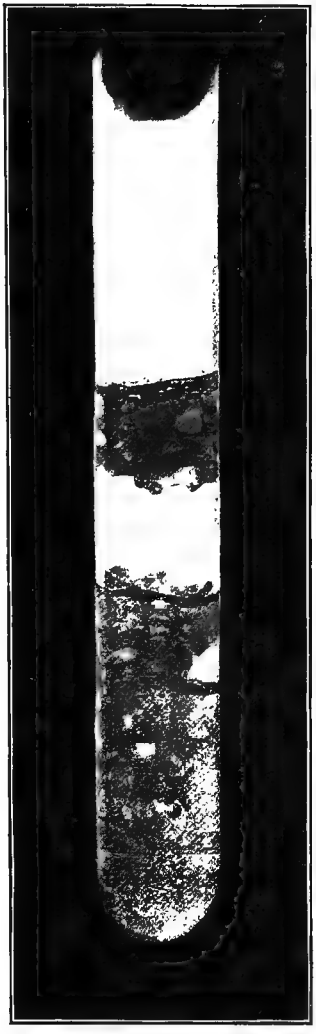

FIG. 73.—BACILLUS AËROGENES CAPSULATUS, CULTURE IN DEXTROSE-AGAR, SHOWING GAS-BUBBLES.

not usually pathogenic for rabbits and mice. In guinea-pigs, sparrows and pigeons it may produce "gas phlegmons." It has been found on numerous occasions in the organs of human cadavers in which a development of gas had taken place, producing bubbles or cavities in the tissues, imparting to them a peculiar spongy character (German, *Schaumorgane*). Probably this is, as a rule, a post-mortem invasion, but there is reason to believe that in some cases it enters the circulation during life. It has been found in cases of emphysematous gangrene or cellulitis, in various uterine infections, including physometra and emphysema of the uterine wall, in pneumothorax and pneumoperitonitis, and in other pathological conditions where gas occurs in the tissues. Exceptionally it may cause pus-formation.* This bacillus, or the gas formed by it in the organs of human cadavers, appears to have furnished the basis for some of the cases in which death has been ascribed to the entrance of air into the veins during life. It is the same as the organism described by E. Fränkel as Bacillus phlegmonis emphysematosæ.

Bacillus œdematis maligni (French, *Vibrion septique*). —A bacillus about 1 μ in breadth, 2 to 10 μ in length, which may form threads, having rounded ends when occurring singly. It is motile, having flagella at the sides and ends. It forms spores, and may bulge at the center in consequence of the spores formed there. It is decolorized by Gram's method. It is a strict anaërobe and is best cultivated under hydrogen. It grows at ordinary temperatures, but better in the incubator. It liquefies gelatin and blood-serum. The colonies in gelatin are spherical and appear like little bubbles. It grows well upon agar. Gas may be produced in these media.

It is found in garden-earth, street dirt and in putrefying organic material. It is pathogenic to rabbits, guinea-pigs,

* Welch. *Philadelphia Medical Journal.* August 4, 1900.

mice, pigeons and various other animals, including man. Inoculation results in the production of swelling and edema, spreading from the point of inoculation. Gas may be produced in the tissue. It may lead to wide-spread septicemia.

Bacillus tetani.—A slim, straight bacillus, with rounded ends, which may form in threads. It is slightly motile. Spores form in culture-media at the end of thirty hours in the incubator. The spores are located at one end, which is

FIG. 74.—TETANUS BACILLI, SHOWING SPORES. (× 1000.)

swollen, so that in this stage the organism has the shape of a drum-stick. The spores are extremely resistant, and in the dry condition remain capable of germinating under favorable conditions for years. They are killed by moist heat at 100° C. in five minutes; by 5 per cent. carbolic acid, in fifteen hours; by bichloride of mercury, 1–1000, in three hours. The tetanus bacillus stains by Gram's method. It is a strict anaërobe; it grows in an atmosphere of hydrogen, but not of carbon dioxide. It may sometimes be made to grow

very well by Buchner's method. It may be cultivated at the room temperature, but better in the incubator. It grows upon ordinary culture-media, preferably those containing dextrose. Gelatin is liquefied slowly; the colonies in gelatin present characteristic radiating filaments and look like a bristle brush. It grows on the other culture-media. Gas formation is not pronounced.

This organism appears to be widely spread in external nature, especially in the soil. It is often found in garden-earth and in the feces of herbivorous animals. McFarland claims that it may occur in vaccine virus when this is carelessly prepared, and this would explain those rare cases of tetanus which occur after vaccination.* Tetanus bacilli have been found in gelatin, and it is stated that tetanus has followed the injection of gelatin as a hemostatic. The infection appears usually, if not always, to be introduced through some wound.† Clinically, persons having the disease suffer from spasms of the muscles about the neck and the lower jaw (lock-jaw). The spasms finally become general.

Inoculation with a pure culture produces tetanus in mice; also in rats, guinea-pigs and rabbits. The tetanic spasms begin in the vicinity of the point of inoculation and afterward become general. The bacilli are not widely scattered through the body; they occur only in the immediate vicinity of the original lesion, and there are no important macroscopic alterations in the internal viscera.

Tetanus is the type of the purely toxic disease. Its symptoms may be produced in animals by the injection of liquid cultures which have been deprived of their bacteria by filtration. The toxic substance appears not to be a ptomaine, as was at first supposed, and its exact nature is not determined.

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* *Journal Medical Research.* Vol. VII. 1902.

† Wells. Fourth of July Tetanus. *American Medicine.* June 13, 1903.

The poison is tremendously powerful (see page 164). It acts as an excitant to the motor cells of the central nervous system, especially the spinal cord. Bolton and Fisch have pointed out the possibility that horses used for the preparation of diphtheria antitoxin may be infected with tetanus, and have tetanus toxin in the blood.*

The activity of the poison is destroyed by heat and by direct sunlight; various chemicals diminish its intensity.

Antitoxin for tetanus has been prepared according to the

FIG. 75.—ANTHRAX BACILLI, FROM A PURE CULTURE.† (× 1000.)

principles employed for antitoxins in general. It has not proved very markedly successful as a curative agent; but as a prophylaxis, where all patients are treated who have deep, dirty wounds, and in a similar way in veterinary practice, it has undoubtedly proved of value. Unfortunately the disease is seldom suspected until a relatively large amount of toxin has

* *Transactions of the Association American Physicians.* 1902.

† The culture was derived from a case of malignant pustule in a tanner. The lesion was excised promptly, and the patient recovered.

formed and begun to manifest its action in the patient's body.*

Bacillus anthracis.—This is the largest of the pathogenic bacteria with the exception of the spirillum of relapsing fever, which is longer but more slender. The bacillus of anthrax is 1.25 μ broad, and from 3 to 10 μ long. Bacillus aërogenes capsulatus is of about the same size. The anthrax bacillus often forms long threads. A capsule is sometimes

FIG. 76.—ANTHRAX BACILLI, SHOWING SPORES. (× 1000.)

present. It is not motile. It forms spores, which are placed in the centers of the bacilli. The spores form only in the presence of oxygen; they do not appear in the body of an infected animal during life. Anthrax spores are the most resistant of all pathogenic bacteria; they have been known to with-

* Moschkowitz. *Studies, Department Pathology. College Physicians and Surgeons.* New York. Vol. VII. 1899–1900. *Annals of Surgery.* P. 442. 1900.

stand boiling for twelve minutes,* 5 per cent. carbolic acid for forty days, and 1–1000 bichloride of mercury for nearly three days. The anthrax bacillus is aërobic, although not strictly so. It stains by Gram's method. It grows at the room temperature, but better in the incubator. It liquefies gelatin and coagulated blood-serum. Colonies in gelatin seen under a low power display numerous, irregular, fine, hair-like projections; stab-cultures in gelatin also present fine projections passing from the needle-puncture into the solid gelatin. It grows on the ordinary culture-media; the growths are usually

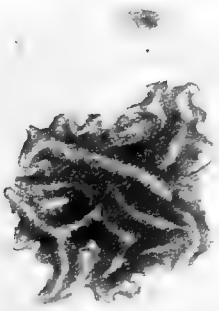

FIG. 77.—COLONY OF ANTHRAX BACILLI (LOW POWER).

whitish. Cultures on potato kept in the incubator are favorable to the development of spores. Milk is coagulated and later peptonized.

It is pathogenic for mice and guinea-pigs, less so for rabbits; it is also pathogenic for sheep and cattle. Rats and pigeons are quite resistant, but not entirely immune; cats, dogs and frogs are not susceptible, or but slightly so.

Anthrax is a disease which occurs chiefly in cattle and sheep. It is commoner on the continent of Europe and in Siberia than

* More than half an hour. V. A. Moore. *Infectious Diseases of Animals.* 1902.

in America. In susceptible animals inoculated with virulent cultures of the anthrax bacillus septicemia is produced. Large numbers of the bacilli are found in the blood, and may be crowded together in the capillaries of the liver and kidneys. Men are occasionally affected, especially those whose occupation brings them in contact with cattle or with the hides and wool of animals that die of the disease. The infection usually occurs through wounds of the skin, where it produces a localized inflammation known as malignant pustule. Anthrax of the lungs or "wool-sorter's" disease may be acquired by inhalation

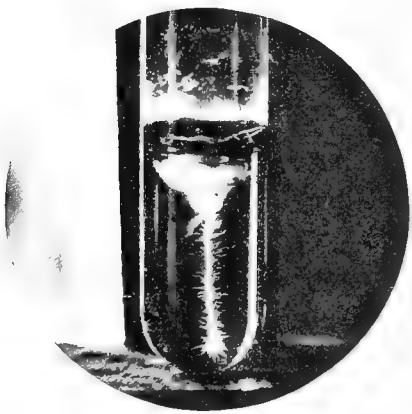

FIG. 78.—BACILLUS OF ANTHRAX. STICK-CULTURE IN GELATIN.—(*Gunther.*)

of material containing the spores of the bacilli. Infection by way of the intestine occurs occasionally but is less common. Laboratory workers engaged in studying the anthrax bacillus have been accidentally infected in a number of instances.

The anthrax bacillus, owing to its large size, was the first of the pathogenic bacteria to be recognized, and its study has furnished the basis for much of our knowledge concerning the infectious diseases. It was for anthrax that Pasteur developed the idea of making a protective vaccine, shortly after he had produced a similar vaccine for chicken cholera. There is

some danger to the inoculated animals attending the use of anthrax vaccines.

In order to obtain material free from spores the blood of an animal which has recently died of anthrax is taken, because anthrax spores do not form in the living body. Cultures made in bouillon are kept at a temperature of from 42° to 43° C. At this temperature spores do not form, while the virulence of the anthrax bacillus becomes gradually diminished. In time the virulence is so far diminished that rabbits will survive

FIG. 79.—ANTHRAX BACILLI WITH SQUARE OR SLIGHTLY CONCAVE ENDS SOMETIMES SEEN; FUCHSIN STAIN. (× 1000.)

inoculation, and eventually also mice and guinea-pigs, which are extremely susceptible to anthrax. Small doses of a culture of extremely weak virulence are given to the animals which it is desired to protect, like cattle and sheep, and subsequently a somewhat more virulent culture is employed.* The method is never used in human beings.

Bacillus influenzæ.—A small bacillus, 0.2 to 0.3 μ by 0.5 μ, with rounded ends. It does not form spores, is not motile

* For details as to the results of this method see V. A. Moore. *Infectious Diseases of Animals.* 1902. For other and unique researches on immunity for anthrax see Emmerich. *Centralblatt für Bakteriologie.* Original. Bd. XXXII. P. 821.

and is decolorized by Gram's method. It is aërobic, grows only in the incubator, and upon media containing hemoglobin. The medium is prepared by smearing sterile blood over the surface of a tube of agar. Fresh, uncoagulated blood may, with care, be mixed with melted agar sufficiently cooled; the mixture may be poured into tubes and slanted; the tubes should be tested in the incubator before using. The blood of some animals, as the pigeon and rabbit, may be used instead

FIG 80.—ANTHRAX BACILLI IN THE CAPILLARIES OF THE LIVER OF A MOUSE, SKETCHED FROM A SECTION STAINED WITH FUCHSIN.

of human blood.* The colonies are small and transparent, looking like little drops of water, not becoming confluent.

Of a large number of bacilli, the majority are destroyed in twenty-four hours or less by drying. They die out in a similar manner in water. Experiments upon animals up to this time are not conclusive. For diagnostic purposes, the sputum should be carefully collected in a sterile bottle. If the particles of sputum are likely to have become contaminated, rinse in

* *Centralblatt für Bakteriologie.* Bd. XXXII. Original. P. 667.

sterile water. Inoculate on ordinary agar and on blood-agar. The influenza bacillus should grow only on the blood-agar and have the other characters above mentioned. Any organism that grows on both the ordinary and the blood-agar must be rejected. As far as is known, this organism attacks spontaneously only human beings. It probably does not grow outside the body in nature. In cases of influenza it is found in the mucous discharges and in the bronchi and lungs. It is the predominating organism in some cases of bronchitis.* According to Canon, the bacilli may sometimes be found in the blood.

Bacillus diphtheriæ (Klebs-Löffler).—A straight or slightly curved bacillus, usually 1.2 to 2.5 μ in length, with rounded or slightly pointed ends, remarkable for showing irregularities of form, sometimes being club-shaped or spindle-shaped; branching forms have been found.† It is not motile and does not form spores. It retains the stain after Gram's method, but it is best stained with watery solutions of the aniline dyes, especially Löffler's alkaline methylene-blue. Very characteristic pictures are obtained by the method of Neisser:

SOLUTION No. 1.

Methylene-blue	1
Alcohol (96 per cent.)	20
Distilled water	950
Glacial acetic acid	50

SOLUTION No. 2.

Bismarck brown	1
Boiling distilled water	500

Stain the cover-glass preparation which has been fixed in the flame in No. 1 one to three seconds; wash in water; stain in No. 2 three to five seconds; wash in water; mount

* See Lord. *Boston Medical and Surgical Journal.* December 8, 1902.
† Hill. *Journal Medical Research.* Vol. VII. 1902.

as usual. The body of the bacillus is stained pale brown, with dark blue spots, especially at the ends (Fig. 82).

The diphtheria bacillus is peculiar in staining irregularly; certain spots stain more sharply than other portions, and darkly stained spots are likely to occur at the ends. It is a facultative anaërobe. It grows most rapidly in the incubator, and slowly, or not at all, below 20° C. Gelatin is not liquefied. It may be cultivated on various alkaline culture-

FIG. 81.—BACILLUS OF DIPHTHERIA. (×1000.)

media, but grows best on Löffler's blood-serum mixture. On this medium the growth consists of small white or cream-colored, slightly elevated colonies, which may become confluent. The morphology of the bacillus is most characteristic when it is cultivated on blood-serum. It also grows upon glycerin-agar. On potato it produces an invisible growth (see Bacillus of Typhoid Fever). In alkaline bouillon containing dextrose or muscle-sugar the reaction becomes

acid in forty-eight hours. The reaction of the bouillon subsequently becomes alkaline. The growth may form a pellicle over the surface of the bouillon. It has also been successfully cultivated on various media to which egg-albumen has been added.

It is killed by a moist heat at 58° C. in ten minutes. It resists desiccation well.

Bacteriological Diagnosis of Diphtheria.—In many large cities the bacteriological diagnosis of diphtheria is undertaken by

FIG. 82.—BACILLUS OF DIPHTHERIA STAINED BY NEISSER'S METHOD. (× 1000.)

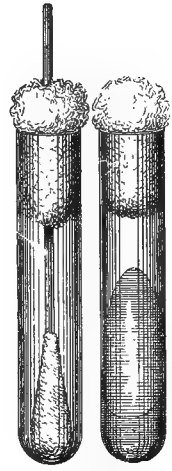

FIG. 83.—SWAB AND CULTURE-TUBE USED IN THE DIAGNOSIS OF DIPHTHERIA.

boards of health. The methods used differ somewhat in detail, but are similar in the main, and are based upon the procedure devised by Biggs and Park for the Board of Health of New York City. Two tubes are furnished in a box. The tubes are like ordinary test-tubes, about three inches in length, rather heavy and without a flange. Both are plugged with cotton. One contains slanted and sterilized Löffler's blood-

serum mixture; the other contains a steel rod, around the lower end of which a pledget of absorbent cotton has been wound and the tube afterwards sterilized. The swab is wiped over the suspected region in the throat, taking care that it touches nothing else, and is then rubbed over the surface of the blood-serum mixture. The swab is returned to its test-tube and the cotton plugs are returned to their respective tubes. The plugs, of course, are held in the fingers during the operation, and care must be taken that the portion of the plug that goes into the tube touches neither the finger nor any other object. The principles, in fact, are the same as those laid down in general for the inoculation of culture-tubes with bacteria (see page 72). In board of health work these tubes are returned to the office. When it is desirable, a second tube may be inoculated from the swab. The tubes are placed in the incubator, where they remain for from twelve to twenty-four hours, and a microscopic examination is then made of smear preparations stained with Löffler's methylene-blue. On Löffler's blood-serum kept in the incubator the bacillus of diphtheria grows more rapidly than the other organisms which are ordi-

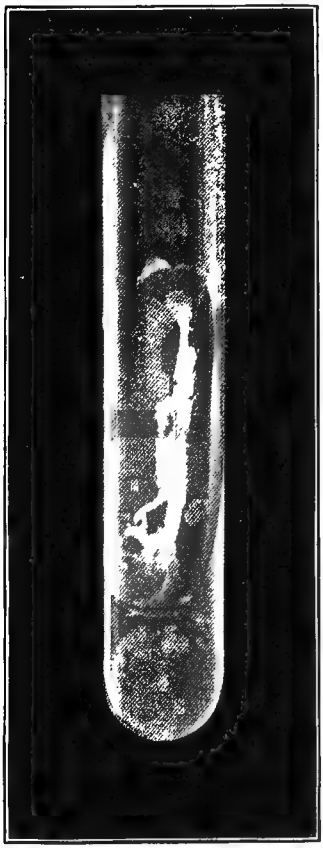

FIG. 84.—BACILLUS OF DIPHTHERIA, CULTURE ON GLYCERIN-AGAR.

narily encountered in the throat, a property which to a certain extent sifts it out, as it were, from them, and makes its recognition with the microscope easy in most cases. The growth, furthermore, is quite characteristic, and its nature can be predicted with considerable accuracy, even without microscopical examination, by one who has had much practice. Colonies of streptococci frequently look very like those of the bacillus of diphtheria, but those two are easily distinguished from each other with the microscope. The diagnosis of the diphtheria bacillus, then, is made from the character of the growth upon the blood-serum and the microscopical examination, taking into account the size and shape of the bacilli, with the frequent occurrence of irregular forms and the peculiar irregularities in staining. In doubtful cases a second culture should be made from the throat.

The very large number of examinations that have been made by various boards of health have shown that the diphtheria bacillus may persist in the throat for a long time—occasionally several weeks after the patient has apparently recovered; also that diphtheria bacilli are occasionally found in the throat when there is an inflammatory condition without any pseudomembrane, and that they sometimes appear in an apparent healthy throat, especially in children who have been associated with cases of diphtheria. It has been found that bacilli sometimes occur in the throat which have all the morphological and cultural properties of the diphtheria bacillus, but which are devoid of virulence when tested upon animals. Such diphtheria bacilli have frequently been called *pseudodiphtheria bacilli.* A bacillus closely resembling the diphtheria bacillus, but without virulence, has been found in xerosis of the conjunctiva. It is called the *xerosis bacillus.* If not a transformed diphtheria bacillus, it is at least closely related. The diphtheria bacillus is subject to wide variations in morphology, so that, in dealing with unknown cultures where the forms are

not characteristic and injection into animals is without result, it may be difficult to decide whether or not the organisms are diphtheria bacilli. Consequently another view with regard to pseudodiphtheria bacilli has arisen. While recognizing that virulent diphtheria bacilli occur, it is also claimed that a distinct *pseudodiphtheria* bacillus exists, different from the diphtheria bacillus, though resembling it. It is shorter, stains more evenly, shows no polar granules by Neisser's method of staining, does not produce acid in dextrose-bouillon, and is not pathogenic to animals. It is found occasionally in the nose and throat and has no connection with diphtheria, according to this view.* But there are some who hold that there is no pseudodiphtheria bacillus, and that the organism so called is merely a more or less modified form of the diphtheria bacillus.

The diphtheria bacillus is pathogenic to animals. When it is injected into them it produces a toxemia. In the guinea-pig, which is especially susceptible, local inflammation results, and death occurs usually in two or three days. The bacilli are found to be confined to the vicinity of the wound, and not usually to be disseminated throughout the whole body. The death of the animal, therefore, is due to the poisons elaborated by the diphtheria bacilli—either poisons introduced at the original injection, or substances produced by the bacilli which may have multiplied in the animal's body. The internal viscera, especially the liver, often exhibit small areas consisting of necrotic cells; a transudation of serum takes place in the great serous cavities, and the lymph-nodes are swollen. A genuine diphtheritic membrane may be produced on the trachea of a young kitten by rubbing into it a part of a culture of the diphtheria bacillus.

* The different sides of this question will be found fully discussed by the following: Wesbrook, Wilson and McDaniel. *Transactions of the Association American Physicians.* 1900. Gorham. *Journal Medical Research.* Vol. VI. 1900. A. Williams. *Ibid.* Vol. VIII. 1902. Denny. *Ibid.* Vol. IX. 1903. Alice Hamilton. *Journal of Infectious Diseases.* Vol. I., No. 4. 1904. Graham Smith. *Journal of Hygiene.* Vol. IV. 1904.

As is well known, the pseudomembranous affection produced by the diphtheria bacillus in man is generally seen in the larynx and pharynx. Membranous rhinitis is also caused by the diphtheria bacillus. On the other hand, pseudomembranous affections of the larynx and pharynx may be produced by streptococci.* Pseudomembranes occurring in the throat during scarlet fever and measles may be due to the diphtheria bacillus, but are more often caused by streptococci. The affection known as membranous croup is usually diphtheria of the larynx, produced by the diphtheria bacillus. The diphtheria bacillus is a rare cause of puerperal fever. Although the uninjured skin is not attacked by the diphtheria bacillus, it may be present in pseudomembranes on wounded surfaces, usually in connection with diphtheria in the throat. Most pseudomembranes formed upon wounds of the skin are produced by other bacteria than the diphtheria bacillus, as is also the case with the pseudomembranous inflammations of the intestines and bladder. Although such inflammations are often called "diphtheritic," it must be remembered that the expression is used in an anatomical sense, meaning that a fibrinous pseudomembrane has formed, extending deeply into the tissues, which is not necessarily caused by the diphtheria bacillus.

In cases of diphtheria in man,† the diphtheria bacillus is generally found limited to the vicinity of the pseudomembrane, and at autopsies it is not usually found in the internal viscera, excepting in the lungs, where diphtheria bacilli may or may not be present when diphtheria is complicated with bronchopneumonia. The general symptoms of the disease, including the paralysis which sometimes follows it, are due to the toxins produced by the bacilli in the throat.

* Bissell. *Medical News.* May 31, 1902. *American Journal Medical Sciences.* February, 1903. Review of Work of Massachusetts Boards of Health.

† For a full study of the lesions of diphtheria see the Monograph of Councilman, Mallory and Pearce. Boston. 1901.

*Diphtheria antitoxin.** It is necessary first to obtain the toxin produced by diphtheria bacilli in a concentrated form. For this purpose virulent diphtheria bacilli are cultivated in alkaline, sugar-free bouillon, in flasks plugged with cotton, exposing a large surface to the air. The bouillon is prepared by leaching out 500 grams of lean chopped beef in 1000 c.c. of water overnight, straining the water off through cheese-cloth, and finally inoculating it with a culture of the colon bacillus. The last-named procedure rids the broth of all muscle-sugar. After adding salt and peptone to the sugar-free beef-juice it is put in wide, flat flasks—Fernbach flasks—and sterilized in the ordinary manner. The cultures are grown in the incubator. After five to ten days they are ready, and are filtered through porcelain.† The filtrate contains the toxin. The toxin is injected into the animal from which the antitoxin is to be obtained in small doses. The dose depends on the strength of the toxin. The animal usually employed is the horse, which should be healthy; the presence of tuberculosis and glanders should have been excluded by testing with tuberculin and mallein; the possible presence of tetanus should also be considered (see page 271).

The injection is repeated at intervals of about one week, using larger and larger doses, until the animal is able to tolerate a very large dose indeed—as much as 300 c.c., or even more. If the treatment is successful, the general condition of the animal should not suffer. The injections last over a long period—usually about two or three months. The general condition of the animal remaining good, the toleration of these large doses of toxin is presumed to indicate the existence of a concentrated antitoxic substance in the blood. Small quantities of blood may be withdrawn from time to time, and the serum tested for its antitoxic strength. When a satisfactory serum has been attained, the animal may be bled and the serum saved for therapeutic purposes. Through an incision in the skin a trocar is inserted into the jugular vein. The blood is drawn into sterilized flasks with every precaution to insure sterility. The blood is allowed to coagulate and is placed for a time in the ice-chest. The serum is then withdrawn with sterilized pipettes. Small amounts of chemical germicides, as carbolic acid or chloroform, are sometimes added to assist in preserving it. This serum is the so-called antitoxin used in medical practice.

Since antitoxin is not obtained as a pure chemical substance, and consequently cannot be weighed and measured as other therapeutic preparations, an arbitrary standard to express the potency of the serum, called an immunity unit, has been devised by Behring and modified by Ehrlich. Formerly this

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* See articles by Park, A. Williams, Atkinson and T. Smith. *Journal of Experimental Medicine.* Vol. I., p. 164; Vol. III., p. 513; Vol. IV., pp. 373 and 649. *Journal Medical Research.* Vol. IX., p. 173.

† W. H. Park adds 10 per cent. of a 5 per cent. solution of carbolic acid to kill the bacilli, and filters through paper on the following day; after adding carbolic acid the Berkefeld filter may be used with advantage instead of filter-paper.

unit was taken to be 10 times that amount of antitoxic serum which just neutralized 10 fatal doses of toxin for guinea-pigs weighing 250 grams. In other words, the exact amount of a certain toxin required to kill a guinea-pig weighing 250 grams in four days having been determined by inoculating a number of guinea-pigs, ten times this amount was put into each of a number of test-tubes, and the antitoxin to be tested was added, a slightly different amount to each tube of toxin. The contents of each tube was then injected into a separate guinea-pig.

If any of the animals survived, the amounts of antitoxin in the tubes with which they had been inoculated having been noted, the smallest of these amounts —*i. e.*, the smallest amount found necessary to neutralize the toxin—was regarded as one-tenth of an antitoxic unit. It was naturally assumed that 10 times this amount of antitoxin would neutralize 100 fatal doses. This, however, was found not to be the case (see Immunity, page 187). So the revised standard now employed in Germany, France, America and other countries is the unit recommended by Ehrlich. This consists of comparing the antitoxin to be standardized with antitoxin specially prepared by Ehrlich for the purpose. This antitoxin of Ehrlich is supplied to the various public and private institutions where antitoxin is prepared, and is carefully standardized against very fresh toxin, which therefore contains little toxoid.

The Ehrlich standard antitoxin is really used in the first place to determine the strength of a given toxin, which in turn is used to determine the value of antitoxin to be standardized. The actual method is to mix varying amounts of the toxin to be tested each with one unit of the standard antitoxin, and that mixture which just suffices as proved by experiment to kill a 250-gram guinea-pig in three or four days is designated L+ (see Immunity, page 188); the mixture which is just neutral is called LO. That amount of antitoxin which just neutralizes L+ contains one antitoxic unit according to this method of standardizing.

The injection of guinea-pigs with antitoxin serves the double purpose of determining the potency of the antitoxin and also of determining the presence or absence of pathogenic substances, such as tetanus toxin.

It has been found possible to prepare antitoxin of a high degree of concentration, so that 500 to 1500 units may be contained in a quantity of serum which it is practicable to give at a single hypodermic injection. The large volume of statistics that have been collected from hospitals and from physicians in private practice indicates that the use of diphtheria antitoxin has effected a very great reduction in the mortality from diphtheria.

Bacillus tuberculosis.—A slim bacillus 1.5 to 4 μ in length, which very frequently presents a beaded appearance, owing to its being dotted with bright, shining spots. Branching forms have been described. The tubercle bacillus is considered by some to be a member of the actinomyces group. It is not motile. It has not been proved that spores are formed; nevertheless certain structures, like caseous lymph-nodes, have been shown to be capable of infecting guinea-pigs with tuberculosis, although tubercle bacilli could not be demonstrated

FIG. 85.—BACILLUS TUBERCULOSIS, FROM A PURE CULTURE. (× 1000.)

in them with the microscope. This makes it seem possible that the organisms were present as spores which eluded the microscopical examination. The tubercle bacilli stain with the ordinary aniline dyes and by Gram's method, but they do not take the stains as readily as most other bacteria, and require prolonged exposure to the dye, on warming of the stain. When once stained, however, with aniline-water dyes or carbol-fuchsin, they are not readily decolorized by acids and alcohol, which fact distinguishes them from all other known bacteria

excepting the leprosy bacillus, the smegma bacillus, possibly the bacillus of syphilis (Lustgarten), and certain bacilli found in milk, butter and cow-dung and on various grasses. All of these may resist decolorization by acids or alcohol, and some resist both. They must always be kept in mind in making a diagnosis of tuberculosis. (See pages 33 and 294.) In examining sputum it is particularly important to bear in mind that acid-proof bacilli, resembling tubercle bacilli, have been found in rare cases in gangrene of the lung. But the organisms found in these cases are longer than tubercle bacilli, as a rule, and branch more often, besides being less resistant to decolorization.* The tubercle bacilli appear to owe their peculiar staining properties to fatty substances contained in the bodies of the bacilli. In stained preparations the bacillus usually appears very distinctly beaded, owing to the presence of stained areas which alternate with unstained areas; these unstained areas have been considered by some to be spores.

FIG. 86.—BRANCHING FORM OF TUBERCLE BACILLUS FROM A CULTURE. (× 1000.)

The Bacillus tuberculosis is aërobic. It is cultivated with considerable difficulty—best at about 38° C. It does not grow at a temperature below 29° C., and cannot therefore be cultivated upon gelatin. It grows well upon blood-serum, where the growth becomes visible in from ten to fourteen days in the incubator. It forms a dry, mealy, scaly mass, elevated above the surface, of a grayish-brown color. It also grows upon glycerin-agar; or glycerin-bouillon, on which it forms a pellicle; upon potato; upon milk containing 1 per cent. of agar and upon coagulated egg (see page 69). It is important to have the

* Ophüls. *Journal Medical Research.* Vol. VIII. 1902. Ohlmacher. *Journal American Medical Association.* 1901.

medium moist. It can be cultivated from tuberculous sputum only with great difficulty. It is best to obtain it from the tissues of an animal that has died of tuberculosis, where the tubercle bacilli may be found unmixed with other bacteria. Pieces of tissue should be taken with the precautions necessary to avoid contamination, and should be broken up and rubbed over the surface of the medium. The tubes must be closed with sealing-wax, paraffin or rubber stoppers, or covered with rubber caps, to prevent drying in the incubator. If rubber

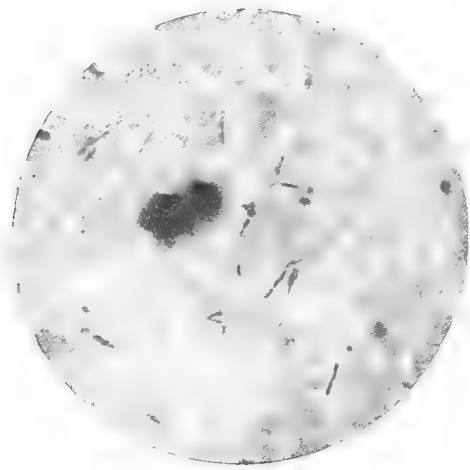

FIG. 87.—BACILLUS TUBERCULOSIS IN SPUTUM, STAINED WITH FUCHSIN AND METHYLENE-BLUE. PHOTOMICROGRAPH IN TWO COLORS. (× 1000.)

caps are used, they should first be left in 1–1000 bichloride of mercury for an hour, and the cotton plug should be burned before putting on the rubber cap. A number of tubes should be inoculated, using rather large particles of the tuberculous material. Among the tubes inoculated, many will fail to present any growth. After the organism has once been grown upon a culture-medium it may be propagated with less difficulty.

It is killed by 5 per cent. solution of carbolic acid in a few minutes. In sputum it is destroyed in twenty-four hours by a three per cent. solution of carbolic acid. It resists desiccation for months, but is killed in some hours by direct sunlight. It is destroyed in a few minutes by boiling.

It is not known to grow, except in artificial cultures, outside of the animal body. It is the cause of tuberculosis in man. It produces tuberculosis in apes, cows, sheep, horses, rabbits, guinea-pigs, cats, field-mice and occasionally in other animals. Guinea-pigs and rabbits are extremely susceptible. A guinea-pig inoculated with tuberculous sputum (provided it does not die of septicemia, due to the pyogenic micrococci which are frequently present in sputum) will present a swelling of the neighboring lymph-nodes in the course of two to four weeks, and will die as a rule in from four to eight weeks, although the time may be longer.

Tuberculosis in cattle (German, *Perlsucht*) is characterized by large, nodular lesions, with a marked tendency to become fibrous, caseous and calcified. The tubercle bacilli of cattle differ somewhat from those of human tuberculosis, as was noted by Theobald Smith.* Whether or not men could be infected with bovine tubercle bacilli has been a question that has been warmly debated in recent years. It seems to have been shown that such infection is possible; also that it is possible that cattle may be infected with human tubercle bacilli. Bovine tubercle bacilli are more virulent for some animals, as rabbits, than human tubercle bacilli.† It seems possible that the danger of infection from cattle has been somewhat overrated.

The lesion produced by the tubercle bacilli in the tissues of men and the lower animals is called a tubercle, which in the beginning is a grayish-white area about the size of a millet-seed. In secretions of the tissue young tubercles are found to present several different structures. Near the

* *Journal Experimental Medicine.* Vol. III., p. 451.

† Theobald Smith. *Medical News.* February 22, 1902. Salmon. Bureau of Animal Industry. Bulletin. No. 33. Adami. *Philadelphia Medical Journal.* February 22, 1902. Ravenel. *University of Pennsylvania Medical Bulletin.* May, 1902. Lartigau. *Journal Medical Research.* Vol. VI. 1901.

center, one or more very large cells called giant-cells occur. They contain several or many nuclei which are frequently arranged in a crescentic manner at one side of the cell. Tubercle bacilli can sometimes be demonstrated inside of the giant-cell. Except possibly in the very youngest tubercles, a small area of necrotic tissue will always be found at the center of the tubercle.

Around the giant-cells and the necrotic area are seen large cells with distinct nuclei which resemble epithelial cells, and are often called epithelioid cells; they are also often termed granulation cells, and represent an attempt at the formation of granulation tissue. But no new-formed blood-vessels, such as are found in granulation tissue as a rule, occur in the tubercle. Tubercle bacilli may also be found among the epithelioid cells. Outside of these epithelioid cells is another layer of small cells called lymphoid cells, which represent leukocytes that have appeared in this situation as a part of the inflammatory reaction excited by the presence of the tubercles. The zone of lymphoid cells may be very indistinct or wanting. Frequently it may be very difficult to make out that the cells are arranged in distinct zones at all. The cells are imbedded in a matrix consisting of the connective tissue originally belonging to the part, to which some fibrin may be added. In addition to the fact that no new blood-vessels are formed to maintain the nutrition of these newly formed cells, the small vessels included in the tubercle and around it suffer from inflammatory changes. Owing to these causes and to a toxic substance formed by or in the tubercle bacilli, degenerative changes and necrosis take place at the central part of the tubercle. As a result of these degenerative changes the center of the tubercle becomes converted into a dry, yellowish-white, friable mass, resembling dry cream-cheese. Such material is said to be caseous, and the process is called *caseation*. Prudden and Hodenpyl found that the injection of dead tubercle

bacilli into animals produced lesions having the histological characters of tubercles, but caseation did not take place.

The small tubercles first formed are called *gray* or *miliary tubercles*. As they become larger they also frequently become confluent. The larger, confluent, caseous tubercles are often called *yellow tubercles*. Swollen tuberculous lymph-nodes of the neck are among the manifestations of the condition formerly known as *scrofula*.

Masses of caseous tubercles sometimes undergo softening. In the lungs the discharge of the softened material results in the formation of a cavity. This formation of a cavity in the lungs is frequently, if not usually, accompanied by secondary infection with pyogenic micrococci. Caseous tuberculous masses may become partly calcified. Very often they may be encapsulated by new formed fibrous or scar tissue. It is possible for tuberculosis to become cured for all practical purposes by means of this process. Autopsies on human subjects have shown that such cures not rarely take place, especially in tuberculosis of the lungs occurring over a localized area. The statistics of autopsies vary widely as to the number of persons that at some time of life suffer from tuberculosis (25 or 30 per cent. and upward). When a tuberculous area has become caseous and encapsulated and apparently quiescent, it is possible for it to be excited to renewed activity under suitable conditions, and, owing to the softening and the discharge of infected material into one of the vessels or cavities of the body, a wide-spreading and rapidly fatal tuberculosis may follow.

Tuberculosis may become disseminated throughout the body from a small focus as a starting-point. The tubercle bacilli may travel through the lymph-spaces and affect adjacent tissues, some of them reaching the nearest group of lymph-nodes. In tuberculosis of the lungs it is usual also to find tubercles in the bronchial lymph-nodes, and in tuberculosis

of the intestines there is also tuberculosis of the mesenteric lymph-nodes. The disease may travel along the serous surfaces and become widely scattered throughout a cavity like that of the pleura or peritoneum. The bacilli may be expelled on some mucous surface and be carried along it to infect some point farther on, as happens when the larynx becomes infected in tuberculosis of the lungs, and when in the same disease tuberculous sputum is swallowed and leads to infection of the intestines. Finally, the infectious material may enter the blood-vessels, especially the veins, and be swept along with the blood-current to become scattered generally throughout the body. In such cases we are likely to have general or *acute miliary tuberculosis.* Almost every organ of the human body may be infected by tuberculosis. Among the most common may be mentioned the lungs, the lymph-nodes, the bones, the intestines, the skin, the meninges, and the serous membranes.

Infection, as far as we know, is always to be attributed directly or indirectly to some preëxisting case of tuberculosis in man or the lower animals. The entrance into the body is most commonly by way of the lungs, where also tuberculous disease is commonest in man, going by the name of *consumption.* This is doubtless due to the prevalent habit of expectorating in public places. Out of fifty-six samples of sputum collected in street cars by Dr. W. G. Bissell, City Bacteriologist in Buffalo, four were tuberculous. In forty-eight samples taken from the floors of a public building by Dr. C. R. Orr, of the pathological laboratory of the University of Buffalo, tubercle bacilli were found three times. According to the researches of Nuttall, a person suffering from tuberculosis may expectorate many millions of tubercle bacilli in the course of twenty-four hours. Coughing and similar efforts may serve to disseminate the bacilli (see page 152).

Concerning the occurrence of tubercle bacilli in cow's milk and butter, see pages 137 and 138.

Cases have been recorded in which the disease was transmitted from the mother to the child in the uterus; how frequently this happens is uncertain. It is usual to attribute greater importance to an inherited tendency to tuberculosis than to the inheritance of the tubercle bacilli themselves.

Agglutination of the tubercle bacillus is said to occur with the serum of cases of tuberculosis under certain circumstances. The reaction does not seem likely to be of practical value.

Tuberculin is made by concentrating a culture of tubercle bacilli grown in glycerin-bouillon to one-tenth of its original volume, over a water-bath, and filtering through an unglazed porcelain filter. It therefore represents the products of tubercle bacilli. It was proposed by Koch as a remedy for tuberculosis, but it has not met with great success, and is little used as a therapeutic agent. It has been found, however, of great value in the diagnosis of tuberculosis, especially in cattle. When tuberculin is injected into a tuberculous animal there results considerable general disturbance, of which the most noticeable evidence is a sudden rise in temperature, while hyperemia is excited around the tuberculous area. In a healthy subject the injection produces no reaction. There is danger attending its use, so that its application in diagnosis is practically confined to cattle.* As a diagnostic measure in cattle it has been found accurate in the great majority of cases. Concerning tuberculosis in cows, see page 137. Supposing that some curative principle exists in the bodies of the tubercle bacilli themselves which could not be procured from cultures deprived of their bacilli by filtration through porcelain, Koch has recently proposed a new form of tuberculin called "tuberculin R," which consists of an extract made from dried and pulverized living tubercle bacilli. The value of this new tuberculin as a remedy is at least doubtful, and physicians are disposed to regard it with a great deal of skepticism.

* For details as to its use in cattle see V. A. Moore. Infectious Diseases of Animals. P. 151. 1902.

Immunity from tuberculosis has been attained experimentally to a certain degree. In very old cultures the virulence of tubercle bacilli sometimes becomes greatly diminished. Animals which survive injections of such bacilli may afterwards withstand large doses of virulent bacilli.*

Acid-proof bacilli resembling tubercle bacilli have been alluded to a number of times (pages 138, 143 and 287). A number of such bacilli have been cultivated, such as those of butter and grass. Injected into animals they may produce nodules more or less like tubercles. In these nodules they sometimes assume forms resembling the fungus of actinomycosis. The tubercle bacillus rarely shows similar forms. All the bacilli of this class, including the tubercle bacillus, sometimes show branching. It is probable that the bacilli of this group are related to the fungus of actinomycosis.† Similar organisms have been found in fishes, in whom they produce nodules resembling tubercles; it is quite possible that the latter organisms are tubercle bacilli, which have been modified by an altered environment. Another acid-proof bacillus has been found which is pathogenic to rats, producing lesions of the skin with nodules; the disease appears in wild rats in certain localities.

Tuberculosis of Birds.—Fowls, ducks and other birds sometimes suffer from tuberculosis due to a bacillus closely resembling the tubercle bacillus of mammals. It has similar staining properties. It sometimes grows in long, branching forms. It differs somewhat from the tubercle bacillus of mammals in its cultural properties. The liver is the organ most often affected. Guinea-pigs are much less susceptible to it than to mammalian tuberculosis. Rabbits are somewhat susceptible, though less so than to mammalian tuberculosis.

Pseudotuberculosis.—Guinea-pigs and other rodents sometimes present lesions macroscopically very similar to those of tuberculosis, in which, however, the tubercle bacilli cannot be found. These affections appear not to be tuberculosis at all, and their nature is not well understood. Several organisms have been found in them, all of which are entirely unlike the tubercle bacillus.

Bacillus lepræ (bacillus of leprosy).—A slim bacillus about 4 μ in length. It is probably not motile. It is uncertain whether or not it forms spores. It stains by the Gram and the Weigert fibrin method, and it is also colored by the methods used for staining the tubercle bacillus. It takes the dye, however, more readily than the tubercle bacillus. In stained preparations it appears very similar to the tubercle bacillus, and re-

* Trudeau. *New York Medical Journal.* July 18, 1903. Salmon. *Philadelphia Medical Journal.* June 13, 1903.

† Abbott and Gildersleeve. *University of Pennsylvania Medical Bulletin.* June, 1902

sembles it in having alternate colored and unstained spots. Although several observers have reported success in attempts to cultivate the bacillus of leprosy, their claims have been disputed. Rost* claims to have succeeded in cultivating the lepra bacillus upon a peculiar medium consisting of a distillate of beef-extract. The distillation is performed by a special arrangement in the autoclave as follows:

Pieces of pumice stone dried in the sun and sterilized are saturated with a solution of beef-extract and transferred to a wide-mouthed jar. The jar is tightly corked, and has two tubes inserted through the cork, one running to near the bottom and opening just outside the cork: the other tube opens near the top of the jar and the end projecting out of the cork is bent to an elbow and is brought through an opening in the autoclave. The end of this tube which is in the jar terminates in a rectangular $\frac{|}{|\,|}$ shaped extremity. When the autoclave is set going, the steam passes into the jar through the tube first described, impinges on the saturated pumice stone, extracts certain substances from the latter, and passes out through the other tube, out of the autoclave, where it is condensed by suitable arrangements. This distillate forms the basis of Rost's various media for the lepra bacillus. The organism is said to grow only in the absence of every trace of sodium chloride.

Rost has also prepared a substance analogous to tuberculin from cultures of lepra bacilli. This he calls leprolin, and it consists of a glycerin extract of a culture grown for three weeks at body temperature. The culture is first evaporated to one-tenth the original volume in vacuo over sulphuric acid, and equal amounts of glycerin added. Subcutaneous injection of 5 c.c. of leprolin is said to cause a temperature of 104° F. in twenty-four hours in a person affected with leprosy, and is being used for diagnostic purposes and also as a therapeutic agent. Its use, however, for either purpose is yet in the experimental stage.

* *Indian Medical Gazette.* Vol. XXXIX. 1904.

The results of inoculation into man and the lower animals of material coming from cases of leprosy have been uncertain. The bacillus of leprosy has been found so constantly in the tissues of those having the disease that it is generally admitted to be the specific cause. The skin and the peripheral nerves are the parts most affected, although other tissues and the internal viscera may be involved. A granulation tissue, forming nodules and thickenings, appears in the affected parts. The bacilli are found in large numbers in the nodules, partly outside of the cells, but mostly within the cells. It is still uncertain whether or not the disease can be transmitted directly from one individual to another, in extra-uterine life, or whether it can be inherited from the parents. However, no explanation can be given for the appearance of the infection in any patient, except communication with some other case. Transmission by contact seems at any rate not to take place easily.

Bacillus mallei (bacillus of glanders).—A slim bacillus with round or pointed ends, which often shows alternate light and dark spots in stained preparations. Branching forms have been described. It is not motile. It probably does not form spores. It does not retain the stain by Gram's method. After staining with the ordinary aniline dyes it is easily decolorized, and on that account it is difficult to demonstrate in sections of tissues. It is facultative anaërobic. It grows at the room temperature, but better in the incubator. It grows slowly on gelatin, and does not liquefy it, or only after a long time. On agar it produces a moist, white growth; on blood-serum, a yellowish or brownish growth; blood-serum is not liquefied. Milk is coagulated slowly, and the reaction becomes acid. On potato the growth is characteristic in one or two days in the incubator, becoming translucent amber-yellow, later a reddish brown, while the surface of the potato becomes discolored.

It is killed in five minutes by a 5 per cent. solution of carbolic acid; in two minutes by 1–5000 bichloride of mercury. It may survive drying for a number of weeks.

In the horse and ass it produces the disease known as glanders, which affects the mucous membrane of the nasal cavity. When the skin is involved, the disease goes by the name of farcy. In the nose, nodules appear in the mucous membrane which become necrotic, forming ulcers. They may become confluent, and may extend along the adjacent surfaces as far as the lungs. There is a profuse discharge from the nose. The neighboring lymph-nodes become involved and are swollen, and nodules may be present in the internal viscera. In the skin the nodes lying underneath the skin are called farcy-buds. Histologically the nodules consist of a granulation tissue, but they tend to break down rapidly, and the process in some respects is very like ordinary suppuration.

This bacillus is pathogenic* for guinea-pigs, field-mice and cats; rabbits, sheep and dogs are less susceptible or only slightly so; also white and house-mice, and hogs; cattle are immune. Men are occasionally infected, especially those who come much in contact with horses. The mucous membranes of the nasal cavity may be the part involved, or the skin or the internal viscera. In a number of instances, workers in the laboratory have been accidentally infected.

The diagnosis of the disease is best effected by the inoculation of a male guinea-pig with the material from a case suspected of being glanders, introducing it into the peritoneal cavity (method of Straus). In about two to three days after an inoculation of this kind there appears a characteristic swelling of the testicle, indicating the beginning of suppuration, which presently takes place; the animal usually dies after two or more weeks. At least two guinea-pigs should be inoculated; and the test may sometimes fail, when it should be repeated on other guinea-pigs.†

* The statements of different writers differ considerably with regard to some of these animals.

† Frothingham. *Journal Medical Research.* Vol. VI. 1901.

Mallein is a product obtained from an old glycerin-bouillon culture of the Bacillus mallei. The cultures are placed in a steam sterilizer for several hours, and are filtered through unglazed portion. The filtrate contains the products of the growth of the Bacillus mallei and is of much the same character as tuberculin. Injected into animals suspected of having glanders, if it produces a local and febrile reaction, the existence of glanders is indicated. This reaction is of use in the diagnosis of the disease in lower animals, especially in horses, where it has been largely employed, though it sometimes fails. An agglutination reaction has been described for the bacillus of glanders.

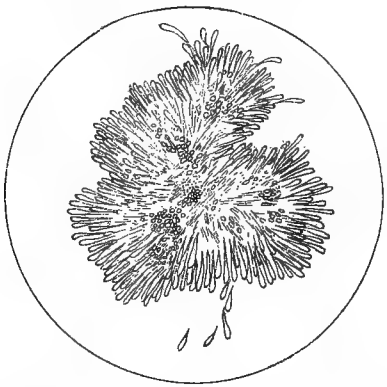

FIG. 88.—RAY-FUNGUS OF ACTINOMYCOSIS. FRESH, UNSTAINED PREPARATION FROM A CASE OF LUMP-JAW IN A COW. (DIAGRAMMATIC.)

Actinomyces bovis* (Streptothrix actinomyces; Ray-fungus of Actinomycosis).—The morphology of this organism is quite different from that of most of the bacteria. It is sometimes considered to be a bacterium of a higher type. The organism appears in the form of threads which show genuine branching. These threads make radiating, interlacing masses. Their external ends are swollen and bulbous under certain conditions. Colonies formed in this manner, seen under moderate magnification, have a radiating appearance which has given rise to the name, ray-fungus. The club-shaped external ends are readily distinguished and the growth possesses a very distinc-

* Hektoen. *Philadelphia Monthly Medical Journal.* November, 1899. Ewing. *Bulletin Johns Hopkins Hospital.* November, 1902.

tive form. This is the shape which the organism presents as it grows in the animal body. The club-shaped ends are generally regarded as a degenerative or involution form. Transverse divisions may sometimes be distinguished upon the threads. Spherical forms resembling micrococci may appear which may possibly be spores. In some members of this group spores—conidia—form in cultures on the ends of the filaments. The organism stains with the ordinary aniline dyes, by Gram's method or the Weigert fibrin stain.

The fungus may be cultivated upon the usual culture-media, though not easily. It is facultative anaërobic. It grows both at ordinary temperatures and in the incubator. The growth is not rapid. The colonies are fine, dry, elevated, irregular in form, becoming opaque. Bulbous ends upon the threads do not usually appear in cultures. The results of the injection of these cultures into the lower animals are as yet uncertain.

The disease produced by the ray-fungus is called actinomycosis. It occurs in cattle chiefly, seldom in swine and horses, and occasionally in man. Infection appears to be carried by grain or particles of vegetable fiber which penetrate the tissue. The presence of such foreign particles as well as the organisms appears to favor infection. The infectious material frequently enters through the mouth, especially in the vicinity of the teeth, but it may also occur through the skin or the mucous membranes. It leads to the formation of inflammatory, tumor-like nodules, hence the name "lump-jaw" given to the disease in cattle. Necrosis of the tissue takes place with the formation of an abscess. The pus is peculiar in containing small yellowish-white particles—so-called "sulphur granules"—which consist of little clumps of the ray-fungus, and which readily permit the disease to be diagnosed by the microscope. The material may be examined in the perfectly fresh condition without any staining. The jaw or its neighborhood

is very frequently affected, or the disease may be present in other situations about the head and neck, and may involve the lungs, the intestines and the vertebræ, ribs and other bones. The disease is usually localized, but a number of areas may be affected simultaneously.

Besides the common actinomyces, there are numerous other ray-fungi, more or less closely related, and whose pathogenic properties are not fully determined. Generally speaking, they appear to be essentially saprophytes, which occasionally become parasitic and pathogenic under especially favorable con-

FIG. 89.—ACTINOMYCES BOVIS, SMEAR PREPARATION FROM A PURE CULTURE, STAINED BY GRAM'S METHOD. (× 1000.)

ditions. A number of species have been found in air, dust, etc., some of them chromogenic. Wolff and Israel described an anaërobic species, pathogenic to man and animals. Madura disease, Madura foot, or mycetoma is a disease occurring in India (rarely elsewhere), affecting one of the extremities, characterized by swellings, nodular deposits and abscesses. Some cases are certainly due to a member of the actinomyces group.*

Other branching organisms, some of them acid-proof, have been described

* Compare Wright. *Journal Experimental Medicine.* Vol. III., p. 421.

chiefly under the name of streptothrix. In man they have been found in a variety of suppurative and necrotic lesions, in particular, bronchopneumonias.*

Bacillus typhosus (Bacillus of Eberth).—A bacillus with rounded ends, varying in length, sometimes making very short, oval forms, sometimes growing out into long threads. It is very actively motile, and possesses numerous flagella which arise from all parts of the surface. It does not form spores. It is not stained by Gram's method, but it may be colored with the ordinary aniline dyes, when the stain will frequently be

FIG. 90.—BACILLUS OF TYPHOID FEVER. (× 1000.)

somewhat irregular. It may be stained in sections of tissues from cases of typhoid fever, with the aniline dyes, such as Löffler's alkaline methylene-blue. It is a facultative anaërobe. It grows at ordinary temperatures, better in the incubator, but grows rather more slowly than B. coli communis. Gelatin is

* Norris and Larkin. *Journal Experimental Medicine.* Vol. V., p. 155. Musser. *Philadelphia Medical Journal.* September 7, 1901. Flexner. *Journal Experimental Medicine.* Vol. III. MacCallum. *Centralblatt für Bakteriologie.* Original. Bd. XXXI. 1902.

not liquefied. Young surface colonies in gelatin appear whitish, with irregular borders and more or less wrinkled surfaces, when slightly magnified. It grows on the ordinary media, and the growths are whitish. Bouillon is clouded. Milk becomes slightly acid, but is not coagulated. In media containing dextrose, acid is formed but no gas. In lactose-bouillon neither acid nor gas is formed, although when grown in milk the typhoid bacilli produce an acid reaction. On the lactose-lit-

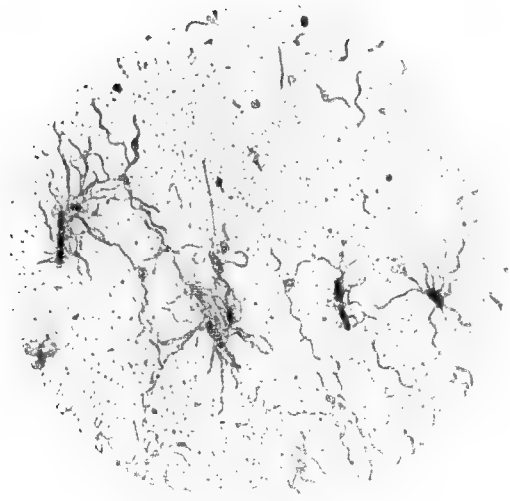

FIG. 91.—BACILLUS OF TYPHOID FEVER, STAINED BY LÖFFLER'S METHOD TO SHOW FLAGELLA. (× 1000.)

mus-gelatin or agar of Wurtz the blue tinge possessed by colonies of the typhoid bacillus on this medium is made use of to distinguish them from colonies of the colon bacillus and other bacteria which form acids from lactose. Neutral red has been used in the same manner, as it is said not to be altered by the typhoid bacillus, but to be changed by the colon bacillus to a yellow color. This medium is prepared by adding to neutral,

plain agar 1 per cent. of a saturated aqueous solution of neutral red, some also add 0.3 per cent. dextrose.

In Dunham's peptone solution indol is not formed, as a rule. On potato it usually forms what is called an invisible growth; that is, although no development is apparent to the eye, numerous bacilli may be shown under the microscope in smear preparations made from the surface of potato inoculated about forty-eight hours previously. Occasionally a slight visible growth is seen on potato.

The typhoid bacillus is killed at 60° C. in ten minutes. It resists drying well. It can survive in soil and sewage a long time.

For a comparison of the properties of the typhoid bacillus and the colon bacillus see the latter.

A new medium has been suggested by Hiss* for the isolation of the typhoid bacillus. It consists of gelatin and agar, beef-extract, sodium chloride and dextrose, and is given a slightly acid reaction. These substances are used in different proportions for plate- and for tube-cultures. This medium is of a semi-solid character, and the great motility of the typhoid bacillus in producing a uniform clouding of the medium in tubes, with the absence of a gas-formation, is made use of to distinguish this organism from the colon bacillus; in plate-cultures the colonies exhibit peculiar filamentous outgrowths. It is claimed that it can be determined whether organisms are typhoid bacilli or not after thirty-six hours in the incubator by this method.

Other special media for the identification of the typhoid bacillus have been devised by Elsner, Stoddart, by Capaldi and Proskauer, and by Piorkowski.† The medium of Stoddart is based upon principles similar to those applied in the medium of Hiss.

The Drigalsky-Conradi‡ method for isolating the B. typhosus from water and feces is that now most employed. The principle of this method consists in the use of a culture-medium on which the surface colonies of B. coli and of

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* *Journal Medical Research.* Vol. VIII. 1902.

† Elsner. *Zeitschrift für Hygiene.* Bd. XXI., p. 25. 1895. Stoddart. *Journal of Pathology and Bacteriology.* Vol. IV., p. 429. 1897. Capaldi and Proskauer. *Zeitschrift für Hygiene,* etc. Bd. XXIII., p. 452. 1896. Piorkowski. *Berliner klinische Wochenschrift.* P. 145. 1899.

‡ Drigalsky-Conradi. Ueber ein Verfahren zum Nachweis der Typhusbacillen. *Zeitschrift für Hygiene und Infectionskrankheiten.* Bd. XXXIX. 1902.

B. typhosus each show a characteristic, macroscopic appearance, so that they can be separated from one another. Furthermore, the medium employed is unfavorable to the growth of many bacteria likely to be present.

In agar containing milk-sugar and blue litmus the colon bacillus causes the formation of red colonies each having a red zone, whereas the typhoid bacillus forms blue colonies.

The addition of crystal violet to the milk-sugar-litmus-agar inhibits the growth of various organisms without materially affecting the growth of B. typhosus. So the medium adopted after many trials was as follows:

(*a*) Three pounds of chipped beef placed overnight in 2 liters of water. Strain off, and boil for one hour, filter and add 20 grams of Witte's peptone, 20 grams of nutrose, and 10 grams of salt. Boil one hour, filter and add to it 60 grams of best stick agar; boil three hours over the flame or one hour in the autoclave, make slightly alkaline to litmus paper, and boil one-half hour.

(*b*) 260 c.c. litmus solution (Kubel and Tiemann); boil for ten minutes; add 30 grams of c. p. milk-sugar, and boil the mixture fifteen minutes.

(*c*) Add solution *b* to the hot, melted solution *a;* mix thoroughly and correct the reaction to weakly alkaline if not already so.

(*d*) Add 4 c.c. of a hot, sterile 10 per cent. solution of dehydrated soda.

(*e*) Add 20 c.c. freshly prepared $\frac{1}{10}$ per cent. solution of crystal violet (Krystallviolet "B," Höchst). This solution should be made with warm, sterilized, distilled water, but not boiled.

A part of this agar is poured into Petri dishes at once. The rest is kept in flasks, about 200 c.c. in each.

The material to be examined is spread over the surface of the plates, not mixed with the medium as is usually done, the object being to obtain surface colonies only.

The spreading is done by means of a glass rod 12 or 14 cm. long, bent at right angles about 3 cm. from one end. The short arm of the bend terminates in a small knob, and is dipped into the material to be examined and run over the surface of the agar in a series of the previously prepared Petri dishes.

Drigalsky-Conradi plates, as described above, are made from water by using the precipitate after centrifuging.

Ficker and Hoffmann* recommend the following method of treating the material for examination for the typhoid bacillus before making the Drigalsky-Conradi plates. They use an enriching fluid which has the property of inhibiting the growth of colon and other contaminating organisms, while not seriously interfering with the growth of the typhoid bacillus.

This fluid consists of:

(*a*) A stock solution of beef-broth. Take one kilogram of chopped beef; add 2 liters of distilled water; heat thirty minutes at 50° to 60° C.; stir; boil

* Ficker and Hoffmann. Weiteres über den Nachweis von Typhusbacillen. *Archiv für Hygiene.* Bd. XLIX. 1904.

for thirty minutes; fill up water lost by evaporation; press through gauze; measure; add 6 per cent. Witte peptone, ½ per cent. salt; heat till peptone dissolves; filter; distribute into sterilized beer bottles with patent stoppers; cover with paper cones; sterilize two hours; clamp the stoppers to, and store away.

(*b*) Enriching fluid: 100 c.c. of the above stock solution measured in a sterilized measuring glass; put in a sterilized Erlenmeyer flask; add sodium hydrate solution, $\frac{n}{1}$, 2.7 c.c. less than the quantity required for phenolphthalein "red point," as determined by neutralizing 25 c.c. Sterilize ten minutes in steam; allow to get cool; add 105 c.c. of a 1.2 per cent. solution of caffeine (solution to be made fresh in cold, sterilized, distilled water every time). Add 1.4 c.c. of a $\frac{1}{10}$ per cent. solution of crystal violet; crystal violet must be dissolved cold.

(*c*) Preparation of the stool:

1. Thin stool allowed to settle, eight-tenths or nine-tenths c.c. of the thin, upper portion added to *b*.

2. Semifluid stool rubbed up in a mortar with 1 part of 1.2 per cent. solution of caffeine; filter through sterilized cotton-wool; eight-tenths or nine-tenths c.c. of the filtrate added to *b*.

3. Thick feces. Rub 1 part of feces with 2 parts of caffeine solution, and proceed as in No. 2.

In all three cases shake thoroughly and place at 37° C.

(*d*) Search for typhoid fever bacillus. Examine a hanging-drop.

1. If there are relatively few bacteria, make 6 large Drigalsky agar plates: plate No. 1 of the series with 0.30 to 0.35 c.c. of the fluid; plate No. 3 with 0.25 c.c.; plate No. 5 with 0.10 c.c. Plates Nos. 2, 4 and 6 are the diluted plates from Nos. 1, 3 and 5.

2. If there is abundant growth, 7 plates are made: 1, 4 and 6 are inoculated with 0.2 c.c., 0.15 c.c. and 0.1 c.c., respectively, and the others are dilutions from these.

Identification of colonies as usual.

Keep the rest of the culture in the enriching fluid on ice. If the first plates fail for any reason, shake this enriching fluid with glass beads and make Drigalsky plates again.

A simpler way of preparing the Drigalsky-Conradi medium is recommended by Hagemann* as follows:

Liebig's extract, 10 grams; Witte's peptone, 10 grams; sodium chloride, 10 grams; water, 600 c.c. Boil in a salt-water bath until 100 c.c. evaporates off. Add 500 c.c. fresh, raw, amphoteric milk. Boil and add agar, 10 grams. Boil until the agar is nearly dissolved; put in the autoclave for twenty to thirty minutes at 110° to 115° C. Filter in the streaming steam. Divide up into sterile Erlenmeyers, about 200 c.c. in each. Sterilize a short time.

* Hagemann. Eine Vereinfachung des Drigalskischen Nahrbodens. *Hygienische Rundschau.* Vol. XIV. 1904.

In using, melt in a water-bath; add normal sodium hydrate till the reaction is slightly alkaline to litmus-paper. Add 20 c.c. Merck's litmus solution. Also add 3 drops of a 1 per cent. alcoholic solution of crystal violet. Mix thoroughly. Pour into Petri dishes, and use as in the original method.

M. W. Richardson has devised an application of the serum-test to plate-colonies suspected of containing typhoid bacilli. If a typhoid colony be torn with a needle, under moderate magnification "a seething motion resembling much the appearance of a swarm of bees" may be seen. This appearance is due to the motility of the bacteria. If such a colony be touched with a small quantity of blood-serum from a case of typhoid fever, the motion is said to cease instantly and almost absolutely. Colonies of other motile bacteria do not undergo a corresponding loss of motility.

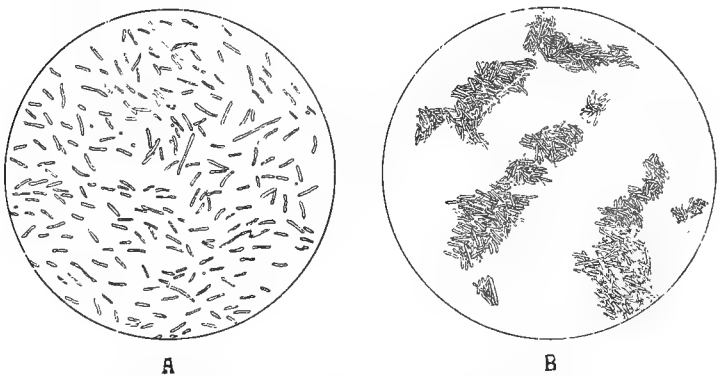

FIG. 92.—APPLICATION OF THE SERUM-REACTION TO TYPHOID BACILLI.

A shows the distribution of the bacilli before the reaction. It is to be remembered that they are motile and their positions may change continually. *B* shows clumping of the motionless bacilli after mixture with the serum of a case of typhoid fever. (Diagrammatic.)

THE SERUM-TEST FOR TYPHOID FEVER.*

When a small quantity of a culture of typhoid bacilli is mixed with a little blood-serum derived from a case of typhoid fever, within a few minutes the motility of the typhoid bacilli ceases and they become agglutinated into clumps or masses (see Agglutinins, Bacterial Poisons, page 166). The bacilli may eventually undergo disintegration into granular material (see Lysins, Bacterial Poisons, page 166). This reaction rarely takes place with the blood-serum of healthy

* This test is often known as the "Widal reaction." For a history and general discussion of the subject see Durham. *Journal of Experimental Medicine.* Vol. V. P. 353.

persons or of those suffering with other diseases, nor when the blood-serum of a typhoid fever case is mixed with motile bacteria other than typhoid bacilli. It has been observed in the blood-serum of an infant born while the mother was convalescing from typhoid fever.

The agglutinating substance has been found in blister-serum and in the milk of typhoid cases, in fluids from the serous cavities and inflammatory and edematous areas in variable amounts, and occasionally in urine, bile and tears.

The reaction may be obtained by adding blood-serum to a young bouillon-culture of typhoid bacilli kept in the incubator, when the occurrence of agglutination becomes manifest by the collection of the bacteria into visible masses or flocculi, which form a sediment. Most investigators prefer to watch the results under the microscope, using an ordinary slide, or, better, the hanging-drop. Young cultures—less than twenty-four hours old—in bouillon, and kept in the incubator, may be used, or, better, cultures kept at room-temperature for twenty-four hours. Johnston and McTaggart recommend that the bouillon cultures be freshly made each time from stock cultures on agar, which need only occasionally be transplanted. Certain stocks of typhoid bacilli seem especially suited to this reaction, and such a stock should be secured.

Blood-serum, blister-serum, fresh blood and dried blood have all been tried with success. Blood dried on unglazed paper or cover-glasses as proposed by Wyatt Johnston is extremely convenient. To perform the test it is mixed with sterilized distilled water, bouillon or normal salt solution; the objection to it lies in the difficulty of securing an accurate dilution. An approximate knowledge of the degree of dilution may be acquired by mixing drops of dried blood of known volume with definite amounts of water, and observing the tints. These should be kept in mind as standards. The dilution may be measured with the hemoglobinometer or with the pipette of the hemocytometer. The New York Board of Health have found blister-serum satisfactory and easy to obtain. A little of the diluted serum is mixed on the cover-glass with a definite amount of the fresh bouillon-culture, and is examined as a hanging-drop. In a short time the characteristic clumping and loss of motility occur. At the same time a drop of the culture alone, and a drop of the culture mixed with *normal serum*, similarly diluted, should be examined as controls. The dilutions used vary from 1 part of serum in 30 to 1 in 50. The higher dilutions are more accurate. The time within which the reaction occurs varies from a few minutes to one or two hours. With little dilution the time should be short; with greater dilution it may be longer. Both cessation of motility and clumping should take place. In a positive case the reaction should be distinct. Normal blood sometimes exhibits agglutinative properties in some degree. If the reaction in any case is not satisfactory, it should be tried with a higher dilution, 1 to 50, and the result should be positive if the case is a genuine case of typhoid fever.

The reaction usually appears between the seventh day and the end of the

third week of the disease; it may be seen earlier; it is often delayed and appears late. The test frequently has to be repeated when the first result is doubtful or negative. Reports indicate that the method is a great aid in the diagnosis of typhoid fever, though not infallible.

Considerable experience is necessary to acquire the judgment needed in using this test.

The agglutinating power becomes lessened after recovery, and usually is wanting at the end of a year. Rarely it may be present for a longer time, a fact that is to be borne in mind in making a diagnosis.

Typhoid bacilli have frequently been obtained from the stools of cases of the disease, but they are isolated only with considerable difficulty. At autopsies they are best cultivated from the spleen, in which, however, it is to be remembered, the Bacillus coli communis may also be present. Puncture of the spleen with a sterilized hypodermic needle, during life, has also been resorted to as a means of diagnosis. The drop of fluid withdrawn may be examined by culture-methods for typhoid bacilli. There is probably some danger to the patient attending this procedure. Cultures made from the blood, where several cubic centimeters are taken, show that a few bacilli occur in the blood in a large proportion of cases of the disease—probably in a majority. Typhoid bacilli appear in the urine in about 20 per cent. of all cases, and the examination of urine for them has been used in diagnosis. The bacilli often occur in the gall-bladder. They have been found associated with gall-stones, and have been supposed to be one of the causes for the formation of gall-stones.* They may remain present in the gall-bladder or in the urine† long after convalescence from the disease. They have been demonstrated in the "rose spots" on the abdomen. They may be present in the lesions of the pneumonia, which frequently complicates typhoid fever, and may appear in the sputum.

Inoculation experiments in animals have not been very

* Pratt. *American Journal Medical Sciences.* Vol. CXXII. 1901.

† M. W. Richardson. *Journal Experimental Medicine.* Vol. IV. 1899.

satisfactory. With a few exceptions, possibly, anatomical lesions resembling those of typhoid fever have not been produced by the inoculation of typhoid bacilli into animals. The injection of cultures into animals may produce death, but it can usually be shown to have resulted from the poisons contained in the cultures.

Typhoid fever is rare during the first two years of life. It frequently attacks young and robust men. The causes that bring about susceptibility to infection are not known.

The principal lesion in typhoid fever lies in the Peyer's patches of the lower part of the small intestines; the mesenteric lymph-nodes and spleen also are swollen. The typhoid bacillus may be demonstrated in sections of the walls of the diseased portions of the intestines. Cases are recorded in which no lesions were found in the intestines, but where the typhoid bacilli were widely spread through the organs of the body, and which therefore represented typhoid septicemia.

Periostitis and osteomyelitis, which are not uncommon sequelæ of typhoid fever, may be caused by typhoid bacilli. Ordinary suppuration may be produced by the typhoid bacillus, but most suppurative affections during or following typhoid fever are mixed infections, or are due to the ordinary pyogenic bacteria.

Typhoid fever is transmitted chiefly through the medium of water, according to present views. It is sometimes conveyed by milk, green vegetables and oysters. Infection through the medium of dust and by the hands and clothing probably occurs, but not commonly. Under certain circumstances the bacilli may be carried by flies.* In caring for cases of typhoid fever the stools, urine, sputum and linen should be disinfected. Persons handling the patient should wash and disinfect their hands:

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* Vaughan. *Philadelphia Medical Journal.* June 9, 1900.

The injection of typhoid bacilli which have been killed by heat has been resorted to as a preventive measure in a large number of cases in the British army. The results appear to have been partially successful, but the method is still in an experimental stage.

Bacillus coli communis (often called simply the *colon bacillus*, Bacterium coli commune of Escherich, and Bacillus pyogenes fœtidus of Passet, who obtained it from foul pus; probably the same as Bacillus Neapolitanus of Emmerich).—A bacillus with rounded ends, frequently of a short, oval form,

FIG. 93.—BACILLUS COLI COMMUNIS. (× 1000.)

when it may be difficult to distinguish from micrococci; often longer, even forming threads. It is slightly motile, having several flagella. It does not form spores. It stains with the ordinary aniline dyes, but not by Gram's method. It is a facultative anaërobe. It grows well at the room-temperature, but more rapidly in the incubator. It does not liquefy gelatin. In gelatin plates the surface colonies are of a bluish-white color; the centers are denser than the borders, which are translucent. It usually grows more rapidly in gelatin than the bacillus of

typhoid fever. Its growths in other media are mostly whitish. Bouillon becomes clouded. Nitrates are reduced to nitrites. In peptone solution it forms indol. On potato it forms an abundant visible growth from cream color to pale brown. Milk becomes acid and is usually, but not always, coagulated slowly. It causes the development of gas and acid in media containing dextrose or lactose. In media containing neutral red it is stated that the colon bacillus produces a yellow color with a green fluorescence. Differential points between

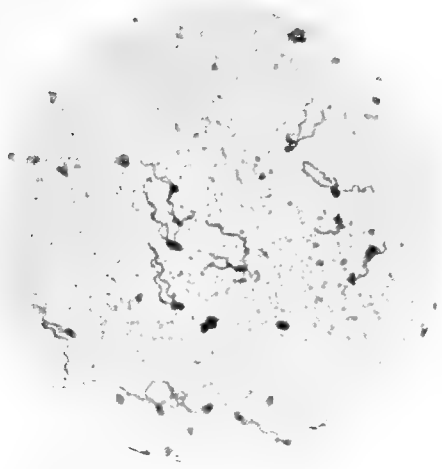


FIG. 94.—BACILLUS COLI COMMUNIS WITH FLAGELLA, STAINED BY VAN ERMENGEM'S METHOD. (× 1000.)

the bacillus of typhoid fever and the Bacillus coli communis are as follows:

1st. The typhoid bacillus is actively motile; the colon bacillus less actively motile.

2d. The typhoid bacillus has numerous flagella which rise from all parts of the surface; the colon bacillus has a smaller number of flagella.

3d. The colonies of the typhoid bacillus in gelatin develop more slowly than those of the colon bacillus.

4th. The superficial colonies of the typhoid bacillus on gelatin plates are less dense than those of the colon bacillus.

5th. In media containing dextrose or lactose the typhoid bacillus does not produce fermentation with gas and the colon bacillus does produce gas in such media.

6th. The typhoid bacillus produces an acid reaction without coagulation in milk, and the colon bacillus produces an acid reaction with coagulation.

7th. In peptone solution the typhoid bacillus, as a rule, produces no indol, and the colon bacillus produces indol.

8th. The typhoid bacillus usually produces an invisible growth on potato, the colon bacillus a visible growth.

9th. The typhoid bacillus is said not to reduce neutral red in media, and the colon bacillus to change it to a yellow color.

To these may be added the growth of the two organisms on special media like those of Wurtz, of Elsner, of Hiss and of Drigalsky and Conradi and the application of the serum-reaction.

Injections of cultures of the Bacillus coli communis into animals produce variable and uncertain results. Subcutaneous injection may lead to pus-formation; in rabbits and guinea-pigs injections may produce death apparently from poisons introduced. With the blood of immunized animals a serum-reaction, similar to that described for typhoid fever, may be demonstrated.

Concerning the occurrence of the Bacillus coli communis in the intestine of man see page 144.*

At autopsies on human subjects the great viscera are often found to have been infected by the colon bacillus, usually when

* See also Moore and Wright. Bacillus coli communis in the Domesticated Animals. *American Medicine.* March 29, 1902.

some lesion of the intestine existed simultaneously, but in most cases without having produced much apparent damage to the organs invaded. The Bacillus coli communis frequently occurs in mixed infections, as in wounds, inflammations and abscesses. It is often found in the peritoneum in peritonitis, in the pus in appendicitis, and in the urine in cystitis; it frequently occurs in the interior of gall-stones with whose formation it may be connected,* as first pointed out by Welch.

There is a large number of more or less closely related organisms which go by the name of the "*colon group.*" The limits of the colon group are extremely ill defined.

Detection of Bacillus coli communis in Water.—To each of a number of fermentation-tubes containing 1 per cent. dextrose-bouillon add some of the suspected water (0.1 to 1 c.c. or more). Place in the incubator. Each day mark the amount of gas that has formed in the closed arm. After two days B. coli communis should render the bouillon strongly acid and produce about 50 per cent. of gas (30 to 70 per cent. according to different writers). The gas is approximately H two parts, and CO_2 one part (see page 120). From tubes showing these characters plates may be made and the usual tests for the colon bacillus applied.† (See Part IV.) Stokes recommends adding the water to fermentation tubes containing 1 per cent. lactose-bouillon and neutral red (10 c.c. of a 5 per cent. solution of neutral red to a liter of bouillon); if the colon bacillus is present, 30 per cent. to 50 per cent. of gas is formed (consisting of one part of carbon dioxide and two parts of hydrogen), and the neutral red in the closed arm changes to a yellow color.‡

Paracolon or paratyphoid bacilli are the names applied to certain members of the colon group which have recently been shown to be pathogenic to man. They may produce clinical symptoms resembling typhoid fever of a mild and atypical form. The affection is rarely fatal. Probably they may occur with typhoid fever in mixed and secondary infections. Characteristic lesions have not yet been observed. The bacilli have been found in the blood, spleen, liver, gall-bladder and urine. Like typhoid and colon bacilli they are motile, have flagella, are not stained by Gram's method and do not liquefy gelatin. They ferment dextrose and maltose, producing acid and gas. They do not ferment lactose. Milk at first becomes acid, later it becomes alkaline, and is not coagulated. On potato a slight visible growth occurs. Media contain-

* Lartigau. *Journal American Medical Association.* April 12, 1902.
† Theobald Smith. *American Journal Medical Sciences.* Vol. CX. 1895.
‡ *Journal of Infectious Diseases.* I. 341.

ing neutral red become yellow, as with B. coli communis, but more slowly, and the red color sometimes returns. In respect to the fermentation of saccharose and the formation of indol reports differ; both are usually negative. The blood of the patient agglutinates the bacilli. But, as among the closely related members of this group mutual reactions are sometimes seen, this test is not to be considered invariable.* Several bacilli allied to the above are known. The Bacillus enteritidis of Gaertner is a related form which has been found in cases of meat-poisoning.

Bacillus lactis aërogenes (Bacillus aërogenes).—A bacillus having a form similar to that of the colon bacillus, described as being larger and plumper. In the main its properties are similar to those of the colon bacillus. Its colonies are more circumscribed and elevated than those of the colon bacillus. It is non-motile. It coagulates milk more rapidly than the colon bacillus. It produces gas upon potato more rapidly than the colon bacillus, and more abundantly. It was first described by Escherich, who discovered the colon bacillus, assigning the Bacillus lactis aërogenes rather to the upper part of the small intestine, and the colon bacillus to the lower portion. According to Kruse, the Bacillus lactis aërogenes and its relatives differ from the Bacillus coli communis chiefly in lacking motility. Like the colon bacillus it has been found many times in the urine in cystitis. See also B. acidi lactici, page 225.

Bacillus dysenteriæ (Shiga).—A bacillus with rounded ends, of the size and shape of typhoid and colon bacilli, seldom forming threads. Most observers have found it non-motile. Vedder and Duval have demonstrated flagella. The bacillus does not form spores. It may be stained with the ordinary aniline dyes; it does not stain by Gram's method. It is a facultative anaërobe. It grows at ordinary temperatures, but better in the incubator. It grows on the usual culture-media, but more

* Cushing. *Bulletin Johns Hopkins Hospital.* July–August, 1900. Strong. *Ibid.* May, 1902. Johnstone, Hewlett and Longcope. *American Journal Medical Sciences.* August, 1902. Libman and Buxton. *Journal Medical Research.* Vol. VIII. 1902.

slowly than B. coli communis. The growths are whitish. Colonies on gelatin plates resemble those of the typhoid bacillus. Bouillon is diffusely clouded; a precipitate may form, but no pellicle. Indol is not produced. Milk becomes acid and is not coagulated. On potato a thin pale layer forms which may become light brown. No gas is formed in media containing glucose or lactose.

Neutral-red agar is not changed. From the feces the bacillus is best cultivated on agar plates, in the incubator. Colonies of B. coli communis are often more numerous than those of the dysentery bacillus. The colonies which develop in twenty-four hours are likely to be colonies of B. coli communis. The position of these may be marked on the glass with a pencil. Those which appear later are to be planted in dextrose-agar. If gas develops, they are not the bacilli of dysentery; otherwise they are to be studied and identified by the cultural and other tests mentioned above, and by the agglutination reaction.

The bacilli are destroyed in a few minutes by boiling, and at 58° C. in half an hour. They appear not to be particularly resistant to the influences that are harmful to bacteria in general.

They have been found in the intestine and the discharges of acute and epidemic dysentery in various climates and countries, including the United States. Thus far their dissemination in the blood and distant organs has not been demonstrated. The lesion of this form of dysentery consists of a severe acute inflammation of the colon, frequently with necrosis of the surface and the formation of pseudomembrane. Ulceration may occur, but is usually superficial. Duval and Bassett found the bacillus of dysentery in the stools of infants having summer diarrhea.

The introduction of pure cultures into animals by way of the alimentary canal has sometimes been followed by a certain amount of diarrhea, but it does not appear that dysen-

tery, as it occurs in man, has been reproduced. Most laboratory animals are, however, very sensitive to the injection into the tissues or veins of cultures, living or dead. They show the lesions produced by various toxins.

The bacillus is agglutinated by the patient's blood, but often only late in the disease and apparently not in all cases. This test seems to have only a limited value in clinical diagnosis. Many prefer to secure the reaction in a test-tube. The dilutions used vary greatly (from 1 in 20 to 1 in 1001.

FIG. 95.—SPIRILLUM OF CHOLERA (× 1000.)

Immunized animals develop the agglutinins in the blood. Results of experiment made for the production of a curative serum are encouraging.

It now seems that the bacillus of Shiga has numerous close congeners, constituting with it a "group." To what extent the others of the group may be concerned in the causation of diarrheal diseases or may occur in the normal intestine is uncertain. According to W. H. Park, some of these form indol and develop acid from mannite which the bacillus of

Shiga does not; they also differ from it in their agglutination reactions.*

Spirillum choleræ Asiaticæ (Comma Bacillus of Cholera).—A rod-shaped organism, somewhat curved, and with pointed ends, hence the name, "comma" bacillus. The curved forms, placed end to end, may produce an S-shaped body. The length is from 0.8 to 2 μ and the breadth from 0.3 to 0.4 μ. In cultures some individuals may develop into genuine spirilla. In the whitish particles found in the stools of cases of cholera the organisms may be present in very large numbers. In these particles they may exhibit a very curious arrangement, lying parallel with one another, and, as remarked by Koch, they resemble a school of fish moving up stream. Involution forms, irregular in outline and staining poorly, are often seen in old cultures. The organism is motile, having a flagellum at one end. It does not form spores. It stains with the ordinary aniline dyes, but not by Gram's method. It is aërobic. It grows at the room-temperature, but better in the incubator. On the ordinary media the growths are whitish. It grows best on neutral or alkaline media, and is very sensitive to a small amount of acid. It liquefies gelatin. The colonies on gelatin plates have a very characteristic appearance. They are nearly round at first, and granular as seen under the low power of the microscope; but at the end of about twenty-four hours the outline is slightly irregular, and the surface looks as though it were covered with finely broken glass. The outline later becomes still more irregular or scalloped. As liquefaction of the gelatin takes place a funnel-shaped depression is formed, into which the colony sinks. Gelatin plates should be kept

* Shiga. *Centralblatt für Bakteriologie.* Bd. XXIV. 1898. Flexner. *Philadelphia Medical Journal.* September 1, 1900. Vedder and Duval. *Journal Experimental Medicine.* Vol. VI. Gay. *University of Pennsylvania Medical Bulletin.* November, 1902. Duval and Bassett. *American Medicine.* Vol. IV. P. 417. 1902. Park and Carey. *Journal Medical Research.* Vol. IX. 1903. Strong and Musgrove. *Journal American Medical Association.* Vol. XXXV. P. 498. 1900.

at a temperature of from 20° to 22° C. In stab-cultures in gelatin a white growth forms around the stab, and at the end of about thirty-six to forty-eight hours a funnel-shaped depression occurs at the surface, owing to the liquefaction of the gelatin. This depression increases in size, and the surface of the liquefied gelatin seems to be surmounted by an air-bubble, which appears to have taken the place of the part of the fluid gelatin which has evaporated. In the deeper portion of the stab liquefaction is less noticeable. The growths on agar are not characteristic.

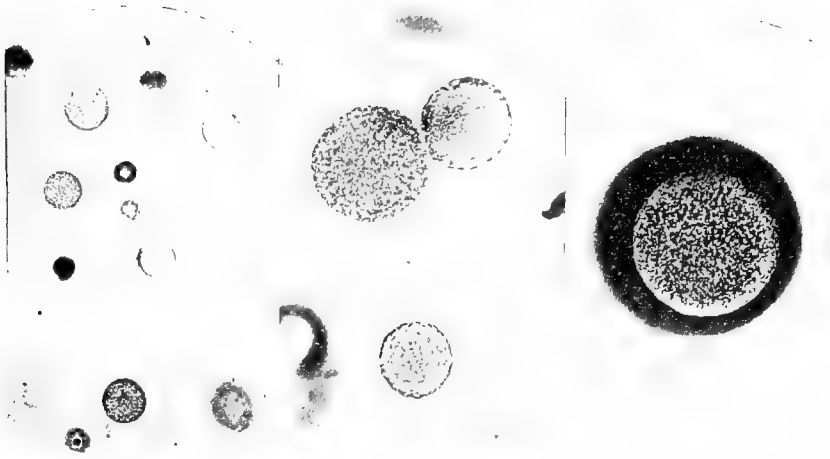
FIG. 96.—INVOLUTION FORMS OF THE SPIRILLUM OF CHOLERA.—(*Van Ermengem.*)

In bouillon a pellicle forms on the surface. On potato in the incubator the growth is whitish or brownish, not conspicuously elevated. After growing it in Dunham's peptone solution in the incubator the addition of sulphuric acid develops a red color, owing to the presence of indol and nitrites,—the so-called "*cholera red*" reaction. Considerable doubt has recently been cast upon the formation of nitrites by the cholera spirillum.* The cholera-red reaction is not confined to this organism, and is said to differ from the nitroso-indol reaction.

The spirillum of cholera is said to be very sensitive to dry-

* Wherry. *Journal of Infectious Diseases.* Vol. II. No. 3. June 24, 1905.

ing, and, provided the drying be complete, is usually killed within twenty-four hours. It is killed in five minutes at a temperature of 65° C. and in one hour at 55° C. It may retain its vitality in water for a long time; observations vary widely in respect to determining how long. In the ordinary food-substances it may survive long enough to allow them to act as carriers of the infection if eaten raw. It is an important fact that the cholera spirillum is not a strict parasite, but under



a. *b.* *c.*

FIG. 97.—SPIRILLUM OF CHOLERA, COLONIES ON GELATIN PLATES. (× 100 TO 150.)—(*Fränkel and Pfeiffer.*)

a. Twenty-four hours old. *b.* Thirty hours old. *c.* Forty-eight hours old.

favorable conditions it may maintain its vitality for some time outside of the human body.

The animals ordinarily used for laboratory experiments are, in their normal condition, not susceptible to infection with the spirillum of cholera through the alimentary canal, and no animal is known which suffers from spontaneous cholera excepting man, though a disease resembling cholera can be reproduced

in animals when certain conditions are complied with. The acid of the gastric juice destroys the organism, and this makes it impossible to infect animals by way of the alimentary tract unless this acidity is overcome with an alkali before the introduction of the culture.

FIG. 98.—SPIRILLUM OF CHOLERA, STAB-CULTURE IN GELATIN, TWO DAYS OLD.—(*Fränkel and Pfeiffer.*)

The following plan was adopted by Koch: The gastric juice was neutralized with a solution of sodium carbonate; the movements of the intestines were quieted by the injection of 1 c.c. of tincture of opium for each 200 grams of the body-weight; and a portion of pure culture of the cholera spirillum was introduced into the stomach. When guinea-pigs are treated in this manner, in most cases a condition closely simulating cholera is produced. The animal dies with symptoms of collapse. The small intestine is more or less filled with a watery, flocculent fluid containing a large number of the spirilla of cholera. The mucous membrane of the intestine is swollen and reddened.

When mice or guinea-pigs receive an intraperitoneal injection from a pure culture, death usually results, apparently from the toxic substances contained in the culture. Pfeiffer was the first to show that an animal may be made immune from cholera by repeated small doses of cultures which have been heated in order to kill the organism. He also showed, in the same connection, that when living comma bacilli are introduced in the peritoneum of an immune animal they first clump together and are then rapidly destroyed and disintegrated (see page 192); furthermore, that a drop of the peritoneal fluid added to a hanging-drop culture of the cholera spirillum produces the same effect. This is now called Pfeiffer's phe-

nomenon, and is the underlying principle of all agglutination reactions, such as the Gruber-Widal typhoid test.

It seems probable, from the results so far obtained, that it is practicable to use injections of attenuated cultures upon human beings with safety, and in this way to protect healthy persons from cholera during an epidemic.*

Although a positive demonstration that the spirillum of Koch is the cause of cholera is lacking, as far as the exact reproduction of the disease in animals is concerned, the necessary proof has been supplied by the accidental or intentional infection of laboratory investigators who were working with cholera, which has happened on several occasions.

Bacteriological investigations have shown that the spirilla of cholera are present in very large numbers in the watery contents of the intestine, especially early in the disease. They appear in the lumina of the glands, and they may be seen underneath the epithelial cells. They may occur in the matters vomited. They usually are not found widely spread through the organs of the body. It is probable that the symptoms of the disease result from poisonous substances produced by the spirilla or contained in them.

The portal of entry in cholera is probably always the alimentary tract, and the infectious agent is usually, though not always, transmitted through drinking-water, and numerous epidemics have been traced to this source. In some cases the origin of the contamination of the water with cholera dejecta has been demonstrated. The organism may, however, be introduced into the alimentary tract upon any and every article of food. It may be conveyed from place to place upon soiled clothing and bedding, and then be brought in contact with food. Flies also probably convey the organisms from cholera stools to articles of food. In order to combat the spread of the disease the excreta and bedding should be thoroughly

* Strong. *American Medicine.* August 15, 1903.

sterilized; the hands of the attendants should be carefully disinfected and all food should be cooked. Although commoner in the summer-time, epidemics of cholera have been known to occur in the winter.

Bacteriological Diagnosis of Cholera.—When cases suspected of being cholera appear in a community, it becomes a matter of the utmost importance to determine the exact nature of the disease in order that it may not become epidemic. One of the first occasions when bacteriological methods were put into practice in the diagnosis of cholera was at the time of the appearance of that disease in the port of New York in 1887.

According to Koch, the diagnosis may be made in twenty-four hours or less. It is important to obtain the discharges from the intestines as early in the course of the disease as possible, and while they are perfectly fresh. It may be necessary, however, to examine the moist dejecta on the linen or clothing, when no other material is available.

In the first place, one of the small, partly solid particles which may be found in the discharges from the intestines should be smeared upon a cover-glass, fixed in the usual manner, stained with one of the aniline dyes, and examined with the microscope. If taken early in the disease, the comma bacilli may be present in large numbers, and they are likely to be arranged in parallel groups, as already described. If comma-shaped bacilli are thus found, a strong probability is created that the disease is Asiatic cholera. The motility of the organisms can be determined by examination in the hanging-drop. It is to be remembered that spirilla of various forms are common in the normal mouth, and may appear in the stools (see pages 141 and 226).

The diagnosis should be confirmed by the use of culture-methods. Using the small, semisolid particles from the intestinal discharges, gelatin plates in the usual three dilu-

tions (see page 85) should be made and kept at a temperature of 20° to 22° C. At the end of twenty-four hours or less the colonies of the spirillum of cholera should have been developed and should present the picture characteristic for these colonies in gelatin plates (Fig. 97), which enables them to be differentiated from colonies of other bacteria. From one of these colonies, preparations may be made for microscopic examination, and a set of tubes may be inoculated. The most characteristic growth will be from stick-cultures in gelatin. The growth in Dunham's peptone solution may be tested for the development of indol and nitrites.

At the time that the first smear preparations and gelatin plates are prepared, tubes of peptone solution should be inoculated directly from the intestinal contents, and kept in the incubator (Schottelius). After development has occurred, the production of indol may be tested by the addition of sulphuric acid. These tubes are especially valuable when unfavorable material or *when material containing small numbers of the spirilla is used.* In the incubator the spirilla may be expected to multiply in the peptone solution rapidly, and to appear upon the surface of the liquid in large numbers, even forming a visible film in six hours. Smears may be made from the surface part of these tubes, stained, and examined with a microscope. From the same material gelatin plates should be prepared, and examined as soon as the colonies develop.

When cultures are obtained, their effects may be tested upon guinea-pigs by injecting them into the peritoneum.

The production of Pfeiffer's phenomenon is an additional means of diagnosis between the cholera spirillum and related forms. This consists in testing the suspected organism with serum from an animal immunized with cultures of cholera bacilli, as already explained above.

In examining suspected water for the spirillum of cholera one or more liters of water is taken, and to it is added enough

of a 20 per cent. peptone solution to make the water contain 1 per cent. peptone, and enough of a 10 per cent. sodium chloride solution to make 5 per cent. The water, with the salt and peptone in it, is divided among a number of sterilized flasks. After twelve hours in the incubator, any cholera spirilla which happen to be present are likely to multiply and form a scum on the surface of the medium, and may be identified according to the methods given above. See also page 131.

Since Koch's discovery of the cholera spirillum in 1883–84 a considerable number of bacteria have been described which resemble the cholera spirillum more or less closely, and these have to be taken into account in making examinations of suspected material of any sort. This is particularly necessary in the investigation of water, in which such cholera-like spirilla seem to occur quite frequently.

Vibrio Metchnikovii.—A comma-shaped organism, which, though somewhat shorter and thicker than the cholera bacillus, is very similar to the latter in form, and, like this, may sometimes form genuine spirilla. It is motile and has a flagellum at one end. It does not form spores. It is aërobic. It stains with the aniline dyes, and is not stained by Gram's method. It grows at the room-temperature. It liquefies gelatin somewhat more rapidly than the spirillum of cholera. The colonies on gelatin plates are not all alike; some of them resemble those of Vibrio proteus, and others are extremely like those of the spirillum of cholera. It grows upon the usual media. Coagulated blood-serum is liquefied by it. The growth on agar is grayish to yellowish, and abundant. It forms a pellicle on bouillon. In milk an acid reaction is developed with coagulation. In peptone solution it produces indol and nitrates like the spirillum of cholera. It is said to give the nitrosoindol reaction more intensely than the spirillum of cholera.

It is killed by a temperature of 50° C. in five minutes. It was discovered in chickens suffering from gastro-enteritis.

It is pathogenic for chickens, pigeons and guinea-pigs; less so for mice and for rabbits. The comma-shaped organisms are found in the blood in guinea-pigs, pigeons and young chickens.

Vibrio proteus (Finkler and Prior).—A comma-shaped organism somewhat larger than the spirillum of cholera, sometimes exhibiting genuine spiral forms, and also, at times, involution forms. It is motile and has a flagellum at one end.

The developments of the colonies in gelatin and the lique-

FIG. 99.—VIBRIO PROTEUS.*

faction of this medium are more rapid than with the cholera spirillum. At the end of twenty-four hours the colonies are all circular, larger than those of the spirillum of cholera, and uniformly granular when slightly magnified. On the other culture-media the growths are usually whitish. On potato it produces an abundant, moist, grayish-yellow deposit, and grows at the room-temperature. It liquefies coagulated blood-serum; milk becomes acid. In peptone solution it does not form indol. It is less pathogenic to animals than the spirillum of cholera.

* The magnification is a little greater than in the other photomicrographs.

It was supposed by its discoverers to be the cause of cholera nostras, but it appears to have no relation to that disease.

Spirillum Milleri.—A comma-shaped organism resembling Vibrio proteus in many respects, and probably identical with it. In gelatin it grows more rapidly, and produces liquefaction more rapidly than the spirillum of cholera. On gelatin plates, at the end of twenty-four hours, the colonies are uniformly circular and granular, lying in little depressions resulting from the liquefaction of the gelatin. Its growths in the other media are not characteristic. It liquefies blood-serum. It does not produce indol. It is less toxic to animals than the spirillum of cholera. It was isolated by Miller from a carious tooth.

See also Spirillum sputigenum, Part III.

Spirillum tyrogenum (Deneke).—A comma-shaped organism, not so large as the spirillum of cholera. It is motile, having a flagellum at one end. It does not form spores. In cultures, genuine spirilla may develop. Gelatin is liquefied more rapidly than by the spirillum of cholera, and the colonies develop more rapidly. The circumference of the colony is round, the surface may appear somewhat granular, and it has a greenish-brown color, seen under the low power. Milk containing litmus becomes acid, is subsequently decolorized, and is also coagulated. It liquefies coagulated blood-serum. It does not form indol in Dunham's peptone solution. No pellicle forms in cultures upon bouillon. It is less toxic to animals than the spirillum of cholera. It was isolated originally from old cheese.

Vibrio Berolinensis.—A comma-shaped organism resembling the spirillum of cholera in form and in the position of its flagellum. It does not stain by Gram's method. It grows at the room-temperature, but more rapidly in the incubator. The colonies upon gelatin, one or two days old, when magnified, are decidedly more finely granular and more transparent than

those of the spirillum of cholera, and the margin is almost absolutely smooth and circular. As the colonies become older they assume a more irregular and lobulated appearance, but are still more finely granular than the colonies of the cholera spirillum. Gelatin is very slowly liquefied. Its growth on the other culture-media is not remarkable. It forms indol in peptone solution, and it increases in the upper layers of the fluid. When guinea-pigs are inoculated in the peritoneal cavity, death

FIG. 100.—SPIRILLUM OF RELAPSING FEVER IN THE BLOOD. SKETCHED FROM A STAINED SPECIMEN.

occurs in one to two days. This organism was discovered in the water-supply of Berlin.

Other spirilla have been isolated from water by Günther (Vibrio aquatilis in Spree water); by Dunbar from the Elbe River; by Russell from the Gulf of Naples; by Heider from the water of the Danube Canal; and in America, by Abbott, from the water of the Schuylkill (Vibrio Schuylkiliensis); and many others have been described to which the limits of this work will not permit of further allusion.

The Spirillum or Spirochæta Obermeieri (of Relapsing Fever).—A slim spirillum with numerous turns, 16 to 40 μ in length. The ends are pointed. It is actively motile. The spirillum is not stained by Gram's method, but may be colored by the ordinary aniline dyes. The organism has never been cultivated. It is found abundantly in the blood and in the spleen during the attack of fever. The spleen is enlarged. The disease has been produced in apes by inoculating them with blood taken from men having the disease.

It is asserted that the spirillum is transferred by bed-bugs from one person to another.*

Spirochæta pallida.—First observed by Schaudinn and Hoffmann† in recent as well as more advanced syphilitic lesions, on the surface and deep in the tissues in chancres, indolent buboes and papules. It has been found by many other observers very recently, and is constantly present in the situations named. The evidence is accumulating rapidly in favor of this organism as the cause of syphilis.‡ It is 4 to 14 μ long, $\frac{1}{4}$ μ thick and has 6 to 14 turns. It is actively motile. Stained with great difficulty. The following stain was recommended originally, and more recently a variety of stains have also been employed by different observers:

(1) Three parts Giemsa's eosin solution (2.5 c.c. 1 per cent. eosin solution in 500 c.c. water).
(2) Three parts asur I solution (1 gram asur in 1000 c.c. water).
(3) Three parts asur II solution (0.8 gram in 1000 water).

Mix and stain dried cover-glass preparations from 16 to 24 hours; wash, dry and mount in balsam.

Spirochæta refringens.—Found less frequently than S. pallida in the same locations as the latter. Is larger and stains more easily than S. pallida.

* Karlinski. *Centralblatt für Bakteriologie.* Bd. XXXI. Original. 1901.
† Schaudinn and Hoffmann. Vorläufiger Bericht über das Vorkommen von Spirochæten in syphililischen Krankheitsprodukten und bei Papillomen. *Arbeiten aus dem Kaiserlichen Gesundheitsamt.* Bd. XXII, p. 527, 1905.
‡ Spirobacteria in the Lesions of Syphilis. *Journal of the American Medical Association.* Vol. XLIV, No. 22, p. 1790. 1905.

APPENDIX.

PATHOGENIC PROTOZOA.

Protozoa are unicellular animal organisms. As they are studied by methods that have much in common with those used for the bacteria they may be considered here briefly. Protozoa are numerous in pond and ditch water, and these species seem to be harmless. However, many diseases of the lower animals are caused by protozoa, such as surra, Texas fever and coccidium disease of rabbits. Birds,* reptiles and frogs† may show organisms in the blood resembling the parasites of malaria. Until recently it has been doubtful whether any pathogenic protozoön has ever been propagated in pure culture outside of the body of the host. This has been accomplished by Novy and MacNeal for a parasite (Trypanosoma) from the blood of the rat ‡ and from many species of birds § on rabbit-blood-agar.

Amœba dysenteriæ (Amœba coli).—Associated with amebic dysentery and believed to be its causative agent is the *Amœba dysenteriæ*, more often named *Amœba coli*. These organisms are found in the intestinal ulcers, the feces, the secondary liver abscesses and the sputum (in the latter only when an amebic liver abscess has perforated into the lung). The lesion in the colon is a severe inflammation accompanied by necrosis chiefly of the submucous layer, and leading to ex-

* Opie and MacCallum. *Journal Experimental Medicine.* Vol. III.

† Langmann. *New York Medical Journal.* January 7, 1899.

‡ Novy and MacNeal. Contributions to Medical Research. Dedicated to Victor C. Vaughan. 1903.

§ Novy and MacNeal. On the Trypanosomes of Birds. *Journal of Infectious Diseases.* Vol. II. No. 2. P. 257. March. 1905.

tensive ulceration.* According to Strong,† at least two distinct species of amebæ have been found in the feces in man, only one of which is pathogenic and the cause of dysentery. Unfortunately the designation, *Amœba coli*, has been applied to both species. The ameba of dysentery should be designated *Amœba dysenteriæ*, limiting the term *Amœba coli* to the non-pathogenic form or forms.

The *Amœba dysenteriæ* is a unicellular organism, 20–50 μ in diameter when at rest, consisting of a clear, homogeneous ectosarc and a granular endosarc, with an eccentrically placed nucleus. The endosarc contains a number of vacuoles of variable size and very frequently red blood-corpuscles, as well as other foreign bodies, such as bacteria, pigment granules, etc. Many red blood-corpuscles may be seen crowded together in a single ameba. The organism is actively ameboid, extending its substance into processes or pseudopodia of varying forms. This ameboid motion assists in making easy the recognition of the parasites under the microscope and in distinguishing them from large, swollen cells found in the feces. The stool should be examined while fresh and still warm.

The non-pathogenic ameba (*Amœba coli*), also occasionally found in the intestinal tract of man, differs from the pathogenic dysenteric organism chiefly in its much smaller size (10–24 μ) and the invariable absence of red corpuscles from its interior. The protoplasmic granules are also, as a rule, smaller and are difficult to recognize. The Amœba dysenteriæ produces experimentally definite ulceration of the gut of cats, whereas the Amœba coli is harmless. Both varieties of amebæ may be stained by a special stain devised by Mallory.‡

* Councilman and Lafleur. *Johns Hopkins Hospital Reports.* Vol. II. Harris. *American Journal Medical Sciences.* Vol. CXV. 1898.

† Strong. Circulars on Tropical Diseases. No. 1. Chief Surgeon's Office, Headquarters, Division of the Philippines, Manila, P. I. February, 1901. *Ibid.* No. 11. April, 1901. (Both reports may be obtained from the United States Government, Washington.)

‡ Mallory. *Journal of Experimental Medicine.* Vol. II. P. 529. September, 1897.

The Malarial Parasite* (Plasmodium or Hæmatozoön malariæ).—The organisms of malaria consist of at least three different species, each associated with one of the three types of malarial fever: The *tertian* parasite with benign tertian malarial fever, the parasite reaching maturity in forty-eight hours; the *quartan* parasite with benign quartan malarial fever, the cycle of development requiring seventy-two hours; and the *estivo-autumnal* parasite with malignant, estivo-autumnal fever, developing to maturity in a variable period of from twenty-four to forty-eight hours. The parasites are studied to best advantage in a drop of fresh, fluid blood placed between a cover-glass and slide and examined with an oil-immersion objective. For method of making and staining dry preparations see pages 44 and 97.

Tertian Parasite.—This appears in its youngest form as a small, round, colorless, hyaline body within the red corpuscle, seen during and just after the chill of the disease. This body may be actively ameboid, suddenly changing its contour into various forms. Its size gradually increases, and fine, dark, actively motile, dancing pigment granules begin to appear at its periphery.

The red corpuscle harboring the parasite, with the growth of the latter, becomes gradually paler and expands in size. The parasite as it grows loses its earlier ameboid movement, and the pigment granules, still actively motile, accumulate. Near the end of forty-eight hours the organism finally fills the red corpuscle, only a faint rim indicating the latter. The ripe parasite now divides it into from fifteen to twenty-five small, round, hyaline spores, which are arranged somewhat radially about the pigment granules which have lost their motility and become concentrated in a clump at the center of

* Thayer and Hewetson. The Malarial Fevers of Baltimore. *Johns Hopkins Hospital Reports.* Vol. V. 1895. Thayer. Lectures on the Malarial Fevers. New York. 1897.

the spore-forming organism. The spores finally break apart and scatter, each destined to invade a red corpuscle and start anew the cycle of development. This cycle may be repeated over and over again, producing a corresponding number of malarial paroxysms.

Fig. 101.

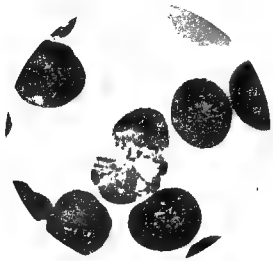


Fig. 102.

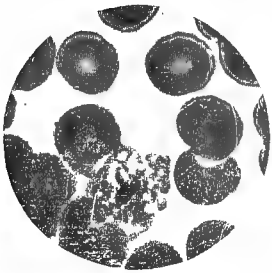


Fig. 103.

Fig. 104.

FIGS. 101–104.—MALARIAL PARASITES IN VARIOUS STAGES. (× 1000.)

Figs. 101, 102 and 103 are tertian parasites. Fig. 103 shows the completion of segmentation. Fig. 104 is the crescentic form of the estivo-autumnal parasite.

Certain full-grown parasites do not complete the cycle of development by sporulation, as described, but, breaking loose from the corpuscle, remain as "*extracellular*" bodies. These are seen chiefly after the paroxysm as large, round, pale bodies containing numerous dancing pigment granules scattered through their substance. They ultimately degenerate and disappear. Some of these extracellular forms may be seen to develop long slender processes, flagella, having a very active whip-like motion. Flagella are never observed

in perfectly fresh blood, but develop only after the blood has been drawn some time, usually fifteen or twenty minutes.

The extracellular forms of the parasite, the *gametes*, incapable of further development in their human *intermediate host*, can continue their life cycle only when, by chance, they happen to be sucked into the body of a mosquito of the genus Anopheles, the *definite host*, in which they undergo a second complete sexual cycle of development with the ultimate production of spores or sporozoïds. When in turn the spores chance to be inoculated into the blood of man by the bite of an infected Anopheles, the man becomes infected, and the cycle of development in the red corpuscle, already outlined, commences. The second or sexual cycle of the parasite in the mosquito, here described for the tertian organism, applies as well to the other varieties of the malarial organism, namely the quartan and the estivo-autumnal forms, in the case of each starting from the extracellular mature forms of the organism found in the blood of the human host.*

Quartan Parasite.—This resembles quite closely the tertian parasite, but differs from it in certain respects. The young, hyaline, intracorpuscular parasite is more highly refractive, its ameboid motion is less marked and more sluggish, and the pigment granules are darker, much coarser, and have very slight motility. The infected red corpuscles are usually somewhat contracted instead of swollen, and their color is apt to be darker, assuming a bronzed hue. The full-grown parasite is much smaller than the corresponding form of the tertian, approximating the size of a normal red corpuscle. As segmentation begins, a characteristic appearance develops which distinguishes the quartan organism, namely, the coarse pigment granules are drawn toward the center of the parasite in certain converging straight paths, giving a stellate arrangement to the pigment, until finally it becomes clumped entirely at the center in a solid mass. The segmenting forms of the quartan parasite thus present a more symmetrical arrangement of the spores, which often resemble the petals of a "marguerite." These spores are oval and number only from six to twelve, being fewer than those of the ter-

* Lyon. The Inoculation of Malaria by the Mosquito. A Review of the Literature. *Medical Record.* February 17, 1900.

tian segmenting parasite. The quartan extracellular forms are smaller than those of the tertian, being about the size of a red corpuscle, and contain coarse pigment granules in active motility until degeneration occurs. Flagella may develop from certain extracellular forms. The entire development of the quartan parasite occupies about seventy-two hours.

Estivo-autumnal Parasite.—This parasite develops to maturity in from twenty-four to forty-eight hours, and is usually regarded as representing a single species, though certain observers claim to distinguish two distinct varieties. The usual description of a single variety is here adopted. The youngest forms (hyaline bodies) resemble those of the tertian and quartan organisms, but are distinctly smaller and more highly refractive. They often present a ring-like appearance. They are ameboid. Pigment granules later appear at their periphery, but are exceedingly minute and scanty, seldom more than one or two being seen. These granules have little or no motility, and in fact are with difficulty made out. The hyaline bodies remain small, seldom exceeding one-third the diameter of a red corpuscle. The infected corpuscle is apt to be crenated, shrunken and dark. These are the forms seen in the circulating blood in early infections; the mature forms, with the exception of the extracellular forms, developing in the spleen and bone-marrow, rarely reach the general circulation. Blood from the spleen shows the full-grown forms in abundance. The segmenting forms resemble those of the tertian parasite both in the numbers of the segments and in their arrangement, but are much smaller in the aggregate, as well as in the individual segments.

After the fever has lasted about one week, extracellular forms make their appearance in the circulating blood. These are crescentic, ovoid or small round bodies, containing coarse pigment granules at their center, generally arranged in a ring. The crescents and ovoid bodies are highly refractive

and are in length about equal to the diameter of a red corpuscle, sometimes larger. The round forms are smaller than a red corpuscle, with the pigment arranged centrally in a ring. They may become flagellated after the blood has remained outside the body for some minutes. Any of the extracellular bodies may show remnants of the red corpuscle attached to its side, like a bib. The extracellular forms are concerned in the cycle of development of the organism in the mosquito, and are sterile in the human body. They are exceedingly resistant to quinine and may continue in the blood for long periods of time.

Melaniferous leukocytes are seen in the blood, being especially abundant after the paroxysm in all forms of malarial infection.* These are phagocytes which have taken up the pigment granules liberated by the disintegration of the erythrocytes.

Small-pox and Vaccinia.—Micrococci of various sorts have been found in the pustules of small-pox and vaccinia, but indicate only a secondary infection. Other microörganisms have been described. The most important are certain bodies often considered protozoa. In both small-pox and vaccinia small, round homogeneous bodies, 2 to 4 μ in diameter, have been found in the epithelial cells of the vesicles. Inoculation of vaccine lymph into the rabbit's cornea leads to the production of similar bodies in the epithelial cells of the cornea. W. Ree† found small ameboid bodies in the blood in cases of small-pox and vaccinia. Vaccine virus that has been filtered through the Chamberland or Berkefeld filter is no longer active. From this it may be presumed that the organism causing it is not too small to be seen with the microscope.

Councilman, Magrath and Brickerhoff,‡ as a result of

* See also Ewing. *Journal Experimental Medicine.* Vols. V. and VI.

† *Journal Experimental Medicine.* Vol. II. P. 515. See also Anna Williams and Flournoy, and W. H. Park. *New York University Bulletin Medical Sciences.* Vol. II. October, 1902.

‡ *Journal Medical Research.* Vol. IX. May, 1903.

recent studies, believe that the bodies above mentioned are protozoa. Segmentation of the bodies is described, resulting in the formation of spore-like bodies. The spore-like bodies undergo a further or second cycle of development within the nucleus. The second cycle also ends in segmentation. The two cycles were seen in small-pox; in vaccinia, only the first or extranuclear bodies were observed.

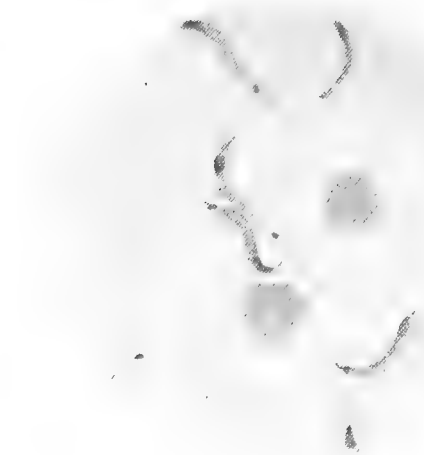


FIG. 105.—TRYPANOSOMES IN THE BLOOD OF THE RAT. ROMANOWSKY STAIN. (× 1000.)

YELLOW FEVER.

It has already been indicated (page 149) that the study of cases of yellow fever has failed to prove that this disease is caused by bacteria. On the other hand, evidence that it is transmitted by the mosquito, Stegomyia, has been increasing.

Trypanosomes.—A number of species of Trypanosoma have been described, which produce diseases in the lower animals; recently one has been stated to be the cause of disease in man.* The trypanosoma is a protozoön belong-

* For a full description of the life history and classification of Trypanosoma see Salmon and Stiles. Emergency Report on Surra. United States Bureau Animal Industry. Bulletin No. 42. 1902. See also Francis. Marine Hospital Service. Hygienic Laboratory. Bulletin No. 11. 1903.

ing to the flagellata. It is of an elongated, spindle-shaped form, with a nucleus, and has a flagellum at one end, which extends along a thin edge, called the undulating membrane. It is actively motile. It occurs in the blood, between, but not in, the blood-corpuscles. Its length is two to several times the diameter of a red corpuscle. Members of this genus are the cause of surra (a fatal disease of horses and mules occurring in India and the Philippine Islands) and of the tsetse-fly disease of South Africa; while others are found in rats, birds, amphibia and fishes. In the horse the infection is transmitted by the bites of flies. Novy and MacNeil have succeeded in cultivating the trypanosoma of rats and birds on rabbit-blood-agar.*

Several cases were reported during 1902 where trypanosomes were found in the blood of individuals from tropical Africa, showing that this group of parasites may occur in man.† The symptomatology of these infections requires further study. Still more recently it has been claimed by Castellani that a trypanosoma is the cause of "sleeping sickness," a disease of the natives of Africa. He states that the parasites may be demonstrated in the cerebrospinal fluid obtained by lumbar puncture and, with greater difficulty, in the blood, during life. Many cases also show at autopsy streptococcus infection, which is believed to be a secondary invasion.‡

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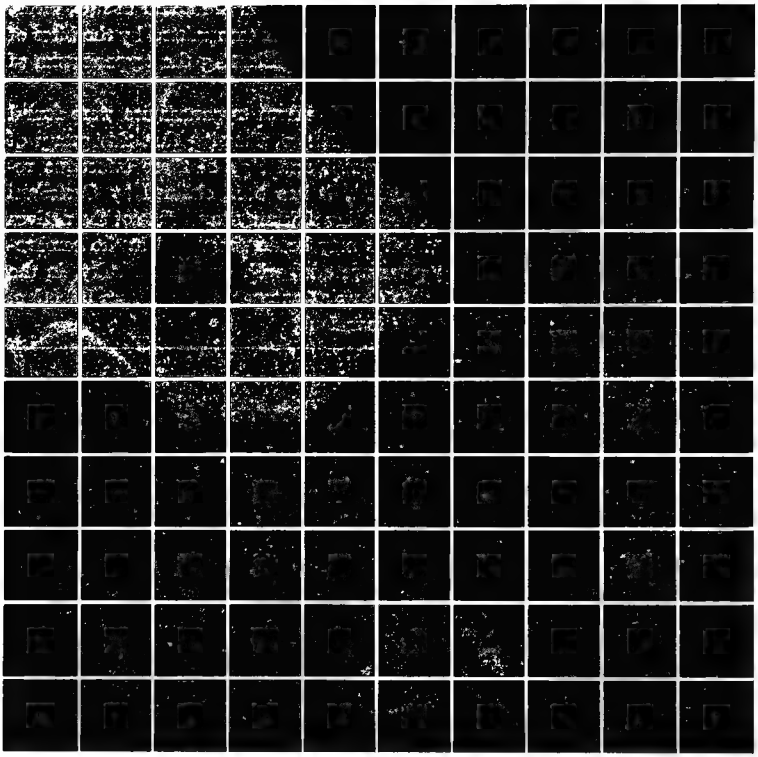
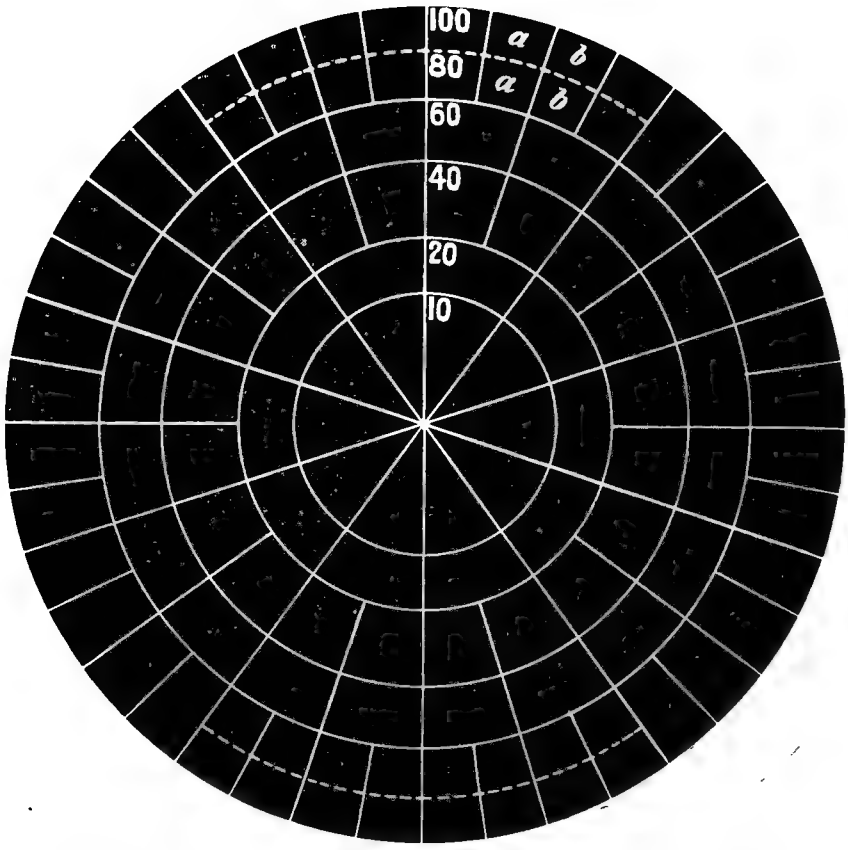
* *Loc. cit.*

† *British Medical Journal.* May 30, 1903.

‡ *British Medical Journal and Lancet.* June 20, 1903.



Surface Divided in Square Centimeters for Counting Colonies.



Jeffer's Plate (Bausch and Lomb). For Counting Colonies of Bacteria on Circular Plates. The Area of Each Division is One Square Centimeter.

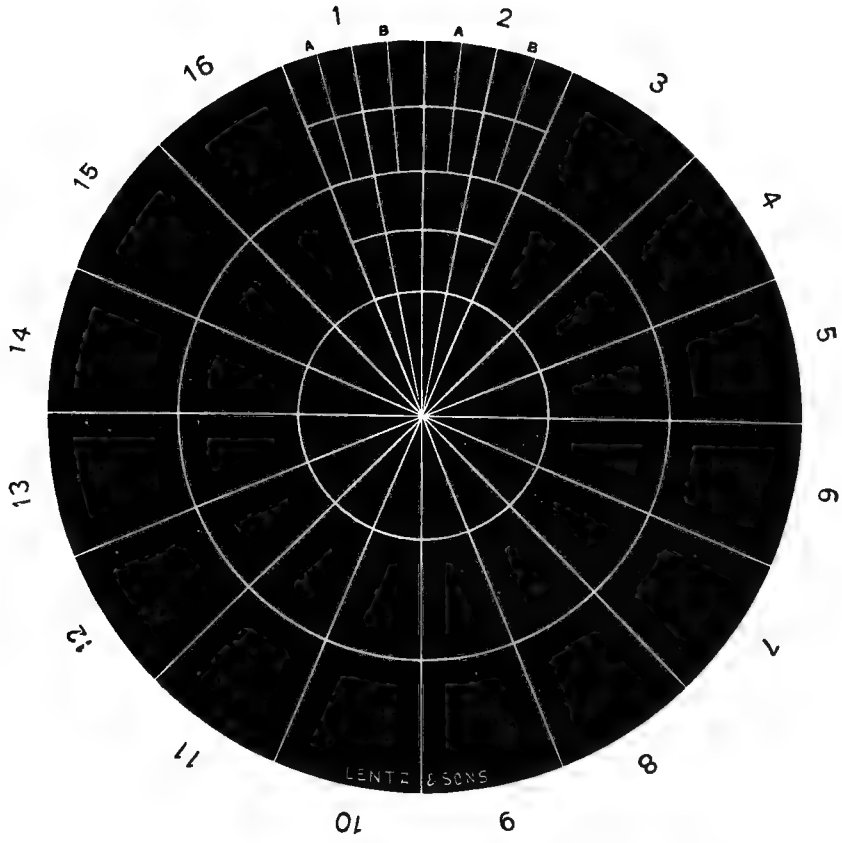


Plate for Counting Colonies of Bacteria in Petri Dishes.

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